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**13. ABSTRACT** *(Maximum 200*

Traumatic brain injury (TBI) contributes to combat morbidity/mortality. We hypothesized that optimal emergency treatment can reduce brain injury in a rat model. Our ultimate goal is translation to the human condition. In yr-1, we studied mechanical ventilation strategies. We found that aggressive hyperventilation early after TBI is detrimental. Also, we developed a model of TBI plus secondary hypoxemia to study therapies, since secondary insults are common. In yr-2, we performed 3 studies, and began a 4th --addressing objectives 2-3. We found that TBI plus secondary hypoxemia was refractory to 4 h of hypothermia--suggesting the need for combination therapies. We also tested prolonged hypothermia (12 h) in our model. Hypothermia improved motor function early after injury. However, by 2 wks, rats treated with hypothermia deteriorated and were ultimately worse (vs normothermia). This suggests the need for studies of hypothermia plus other therapies. We found that the NMDA antagonist MK-801 improved outcome after TBI-- suggesting excitotoxicity as a promising therapeutic target. Fentanyl is used in patients with TBI; but lacks anti-excitotoxic properties. We are evaluating fentanyl in our model. Two fellows worked with the PI, and presented 3 abstracts (2-4). We also published an invited review (6).

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1998 Annual Technical Report

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# INTRODUCTION

In our application, we highlighted the fact that traumatic brain injury (TBI) is an important contributor to combat casualty morbidity and mortality. We also stated that although progress has been made in the development of rodent models of TBI (such as the controlled cortical impact (CCI) model), most of the studies with these models have focused on molecular mechanisms of damage. Because there has been a paucity of application of **practical emergency interventions** in TBI models, we felt that it was important to address this deficiency and that this approach could have important implications for field and emergency management of both soldiers and civilians with severe TBI.

Our overall **hypothesis** is that optimal manipulation of practical interventions applicable in the emergency treatment of severe TBI (respiratory management, temperature control, and sedation) can reduce secondary brain injury in a rat model of brain contusion, and thereby improve functional and/or neuropathological outcome.

In the yr-1 of funding, we addressed the most important aspect of the **first Technical Objective** of our proposal -namely- to perform a comprehensive study of the effects of mechanical ventilation strategies (as applied by the first responder in the field) on both functional and neuropathological outcome in our model. **We found that aggressive, prophylactic hyperventilation (HV) applied for 4 hours immediately after injury is detrimental (vs ventilation to a normal $PaCO_2$), and leads to an increase in the amount of neuronal death in selectively vulnerable brain regions.** This study was published as a full manuscript in the ***Journal of Neurosurgery*** (1). We were pleased that the reviewers indicated that this was an important study that would be cited often.

Also, to set the stage for the evaluation of therapies targeting improvement in outcome after severe TBI (as proposed studies in technical objectives 2-4), it appeared that it would be optimal to have TBI models both with and without a secondary insult since such insults are common in the field. This was done by adding a 30 min period of moderate hypoxemia to the CCI insult. The characterization of that model was described in last year's report and presented this year at the National Neurotrauma Society Meeting (2). As evidenced below, during yr-2, we have used both the standard CCI model and the CCI plus secondary insult model to provide insight on important therapies.

This year we performed three comprehensive studies addressing Technical Objective III and part of Objective II. In addition, we have begun a fourth study. These studies included 1) assessment of the effect of transient (4 h), moderate hypothermia on outcome after TBI with a secondary insult, 2) assessment of the effect of prolonged (12 h) moderate hypothermia on outcome after TBI, 3) assessment of the effect of the application of anti-excitotoxic therapy (the NMDA-receptor antagonist MK-801) early after TBI in our model, and 4) comparison of injury using two different anesthetic regimens (isoflurane or fentanyl [the standard emergency department and ICU sedative]). The results of these studies are summarized below. Finally, two research fellows (Drs. C. Robertson and R. Ruppel) worked on these projects with the PI (Dr. Kochanek) during yr-2. Dr. Robertson presented two abstracts of this work-- at the 1999 annual meeting of the National Neurotrauma Society (2,3) --and will present another abstract at the

Annual Meeting of the Society of Critical Care Medicine (4). That work is currently being prepared in full manuscript form. Also, in related studies, we recently reported that 4 hours of moderate hypothermia attenuates DNA damage assessed at 4 hours after injury using the Klenow method (5). Finally, some of our work on hypothermia in TBI was summarized in an invited review article that we published in a monograph by the International Trauma, Anesthesia and Critical Care Medicine Society (ITACCS)(6).

## (6) BODY

### (a) Technical Objective 1: Mechanical ventilation strategies after severe TBI in rats (see summary for 1996-1997 [yr-1]). Also see reference 1.

**Recommendation**

We showed that aggressive, early HV after TBI augments neuronal death in CA3 hippocampus in a rat model of cerebral contusion. The further reduction of CBF with HV during the low cerebral blood flow state immediately after injury coupled with alkalosis may increase the vulnerability of selected neurons to damage. The results of this study reinforce the meticulous attention necessary to prevent secondary injury after TBI. A risk in the use of HV is demonstrated. Unless signs of impending herniation (unilateral pupillary dilation, hypertension, bradycardia) are present, this study supports the targeting of normocarbia for mechanical ventilation in the emergency stabilization of the brain trauma victim.

### (b) Technical Objectives 2-4: Testing of field-relevant therapies in experimental models of severe TBI (with and without a secondary hypoxemic insult) in rats .

**(b1)** *Effect of transient (4 h), moderate (32°C) hypothermia on functional and histological outcome after experimental TBI with a superimposed secondary hypoxemic insult in rats.*

We tested the effect of 4 hours of hypothermia in our model of TBI with a 30-min secondary hypoxemic insult. Hypothermia has been shown to be effective in a variety of experimental models with transient application (1-4 h) and in humans (32°C applied for 24 h). **However, in neither experimental nor clinical TBI has hypothermia been tested when applied after the combination of TBI with a secondary insult.**

**Method**

All of the protocols for the studies outlined below were approved by the Animal Care and Use Committee of the University of Pittsburgh. Intubated, mechanically ventilated, isoflurane-anesthetized male Sprague-Dawley rats (n = 43) were subjected to CCI (4 m/s, 2.5 mm depth of deformation) followed by a 30 min controlled hypoxemic insult that reproducibly results in a $PaO_2$ of 40-45 mm Hg. Rats were treated with one of the following three regimens—1) Brain temperature maintained at 37°C applied throughout a 5 hours period (n = 19), 2) Brain temperature maintained at 32°C applied for 4 hours beginning after insult (beginning after both TBI and secondary hypoxemia) and then followed by re-warming over 1 hour (n = 14), and 3) Brain temperature maintained at 37°C applied immediately after TBI (before the secondary

hypoxemic insult) and continued for 4 hours and followed by re-warming over 1 hour (n = 10). After 5 h, rats were weaned from mechanical ventilation, extubated and returned to their cages. Beam balance/beam walking and Morris water maze (MWM) performance latencies were measured in eight rats from each group on days 1-5 and 14-20 post CCI, respectively. Rats were killed at 21 d. Serial coronal sections of brain were studied for contusion volume and hippocampal neuron counting [CA1, CA3] by a blinded observer.

## Results

There were no significant differences in recovery of motor function (beam balance, beam walking, Figure 1) tested on days 1-5 after injury or cognitive function (spatial memory acquisition paradigm on the Morris water maze [MWM], Figure 2) tested between days 14-20 after injury. There were also no significant differences in lesion volume or hippocampal neuron counts between groups at 21 days after injury (Table 1). There was a trend toward reduced contusion volume in the immediate post injury group, however, it did not reach statistical significance.

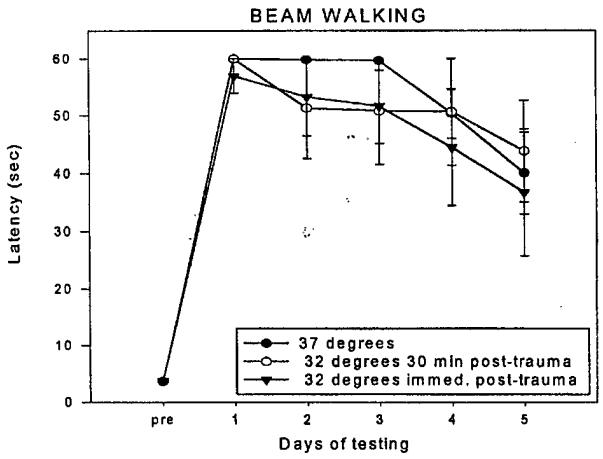


***Figure 1.*** *Effect of hypothermia on motor outcome after experimental TBI plus a secondary hypoxemic insult in rats. Mean beam walking performance latencies (mean ± SEM, in sec) in rats before and on days 1-5 after CCI (4 m/s, 2.5 mm cortical deformation depth). Analysis of variance with repeated measures revealed no difference between the three groups. Data are mean ± SEM.*

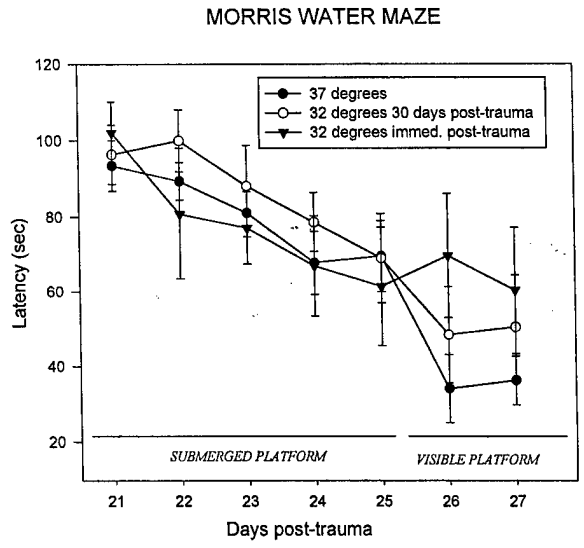


***Figure 2.*** *Effect of hypothermia on cognitive outcome after experimental TBI plus a secondary hypoxemic insult in rats. MWM performance latency to find a hidden platform (mean ± SEM, in sec) by rats on days 14-20 after CCI is depicted. There were no between group differences when performances were compared using ANOVA with repeated measures. Data are mean ± SEM.*

**Table 1. Effect of transient moderate hypothermia on histological outcome at 21 days after experimental TBI with secondary hypoxemic insult in rats.**

| GROUP | Rat survival rate | Contusion Volume | CA3 Survival, mean # neurons per hpf | CA1 Survival, mean # neurons per hpf |
|---|---|---|---|---|
| 37°C | 15/19 (78.95%) | $mm^3$ =65.34±6.94 | 19.8 ± 4.6 | 19.4 ± 4.2 |
| 32 °C, application delayed 30 min until after secondary hypoxemic insult | 8/14 (57.14%) | $mm^3$=53.69±7.93 | 18.5 ± 7.3 | 13.7 ± 5.8 |
| 32 °C, application begun immediately after TBI, before secondary hypoxemic insult | 8/10 (80.00%) | $mm^3$ =50.17±8.23 | 15.6 ± 7.3 | 13.2 ± 8.7 |

All data are mean ± SEM

**Discussion**

Surprisingly, we found that the combined insult of TBI plus secondary hypoxemia was refractory to 4 hours of moderate hypothermia. This is an important finding that was presented in November, 1998 at the annual Meeting of the National Neurotrauma Society, and will be presented in January, 1999 Meeting of the Society of Critical Care Medicine. It suggests the need for combination therapies in this setting. Alternatively, it was possible that the combined TBI plus hypoxemia insult was too severe to favorably effect outcome with any therapy. To address that possibility, we proceeded to perform two studies. These are outlined below.

**(b2)** *Effect of prolonged (12 h), moderate (32°C) hypothermia on functional and histological outcome after experimental TBI in rats.*

The need for combined therapies was suggested, again by the second trial of hypothermia we performed this year. In the second experimental paradigm, we sought to test, to our knowledge for the first time in any laboratory, the prolonged application of hypothermia in a rodent model of TBI. This included over 13 hours of controlled mechanical ventilation and physiological monitoring. To date, only brief 1-4 hours applications have been tested. In this study, we examined TBI without a secondary insult.

**Method**

Intubated, mechanically ventilated, isoflurane-anesthetized male Sprague-Dawley rats (n = 20) were subjected to CCI (4 m/s, 2.5 mm depth of deformation) and treated with one of the following two regimens—1) Brain temperature maintained at 37°C applied throughout a 13 hours period (n = 10),

2) Brain temperature maintained at 32°C applied for 12 hours beginning after insult (beginning after TBI and followed by re-warming over 1 hour [n = 10]). Rats were then weaned from mechanical ventilation, extubated and returned to their cages. They tolerated the procedure remarkably well. Beam balance/walking and MWM performance latencies were measured in all

rats from each group on days 1-5 and 14-20 post CCI, respectively. Rats were killed at 21 days. Serial coronal sections of brain were studied for contusion volume and hippocampal neuron counting [CA1, CA3] by a blinded observer.

## Results

Motor function, as quantified by beam walking task score recovered more rapidly in rats treated with hypothermia. (beam walking p=0.06 vs normothermia, Figure 3A). In contrast, rats deteriorated between 5 and 14 days after injury as reflected by the fact that cognitive function (spatial memory acquisition paradigm on the MWM, Figure 4) tested between days 14-20 after injury was worse in the hypothermia treated group. Histology, from these rats is currently being processed.

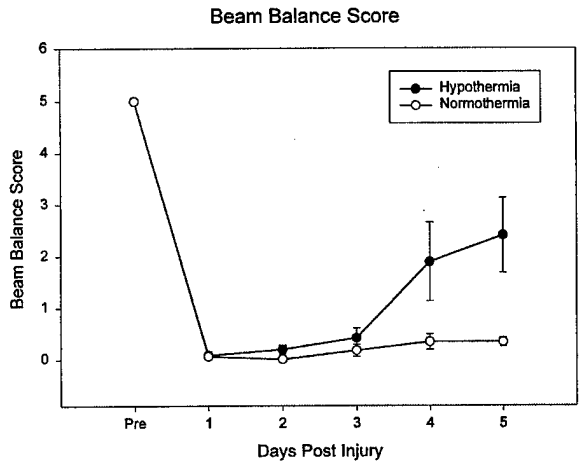


***Figure 3.*** *Effect of prolonged (12 h) of hypothermia on motor outcome after experimental TBI in rats. Mean beam walking score (mean ± SEM, in sec) in rats before and on days 1-5 after CCI (4 m/s, 2.5 mm cortical deformation depth). Analysis of variance with repeated measures revealed a trend toward a significant difference in favor of hypothermia (p=0.06). Data are mean ± SEM.*

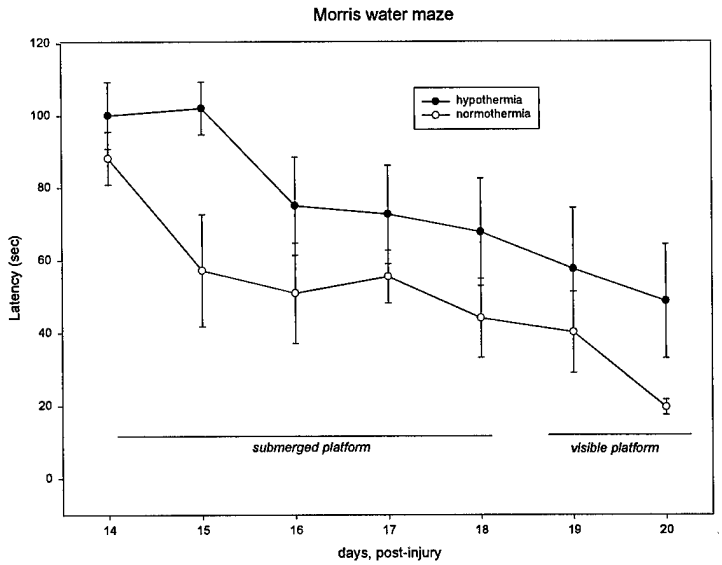


***Figure 4.*** *Effect of prolonged (12 h) hypothermia on cognitive outcome after experimental TBI in rats. MWM performance latency to find a hidden platform (mean ± SEM, in sec) by rats on days 14-20 after CCI is depicted. There was a trend towards a worsening by hypothermia (p=0.082) when treatment groups were compared using ANOVA with repeated measures. Data are mean ± SEM*

**Discussion**

In this demanding experimental paradigm, testing 12 hours of hypothermia, we found that there were beneficial effects of hypothermia on motor function during the initial 5 days after TBI. However, by 2-3 wks after injury, rats treated with hypothermia had deteriorated and their performance on cognitive outcome tasks (MWM) was worse than the group treated with normothermia. One possible explanation for this is the inhibition of nerve growth factor synthesis by hypothermia (previously shown by our co-investigator, S. DeKosky). Thus, acute benefits of hypothermia on mechanisms such as cerebral swelling may be counterbalanced by detrimental effects on "regeneration" or other mechanisms yet to be defined. It is our opinion that this may be an extremely important finding. These data also again strongly suggest the need for studies of hypothermia plus other therapies during and after re-warming. To further strengthen these data, in year 3 we will again compare 12 hours of hypothermia vs normothermia in a squadron of rats, examining its effect on brain edema, intracranial hypertension, and markers of neuronal death (DNA damage) early after in insult (at the completion of the 12 hours period of temperature control). If these markers are favorably affected (as anticipated), it would mirror the clinical condition, and strengthen the relevance of our model for the proposed studies in year 3 (combination treatments). Recently, we demonstrated that 4 hours of hypothermia reduces DNA damage in our CCI model (Whalen et al, Soc for Neurosci Abstract,

**(b3)** *Effect of the anti-excitotoxic NMDA-receptor antagonist MK-801 on functional and histological outcome after experimental TBI in rats.*

In experimental cerebral ischemia, Dietrich et al (*J Cereb Blood Flow Metab* 15:960, 1995) demonstrated efficacy of transient hypothermia plus sustained treatment (for several days after insult) with the anti-excitotoxic agent MK-801. The delayed deterioration after 1 wk in our model seen with the application of hypothermia suggests the possible need for combined therapies. In the third experimental paradigm, we sought to test the effect of the traditional NMDA-receptor antagonist MK-801 in our TBI model (without secondary insult), to set the stage for combination therapies.

**Method**

Intubated, mechanically ventilated, isoflurane-anesthetized male Sprague-Dawley rats (n = 30) were subjected to CCI (4 m/s, 2.5 mm depth of deformation) and treated with one of the following two regimens—1) MK-801 (a single 1 mg/kg IP dose immediately after injury) or vehicle. A separate sham group (all surgery including craniotomy, but no TBI was also studied. Brain temperature maintained at 37°C during TBI. Rats were then weaned from mechanical ventilation, extubated and returned to their cages. They tolerated the procedure remarkably well. Beam balance/walking and Morris water maze (MWM) performance latencies were measured in all rats from each group on days 1-5 and 14-20 post CCI, respectively. Rats were killed at 21 d. Serial coronal sections of brain were studied for contusion volume and hippocampal neuron counting [CA1, CA3] by a blinded observer.

## Results

Motor function, as quantified by both beam balance and beam walking tasks recovered more rapidly in rats treated with MK-801 (Figure 5). MWM performance in MK-801-treated rats did not differ between treatment groups (Figure 6). However, a significantly improved performance in the probe trial (Figure 7) was seen in MK-801 vs vehicle groups. Lesion volume data did not differ between groups (Table 2). There was similar tissue loss in both MK-801 and vehicle treated groups in the injured hemisphere at 21 days after injury. Hippocampal cell counts are still being processed.

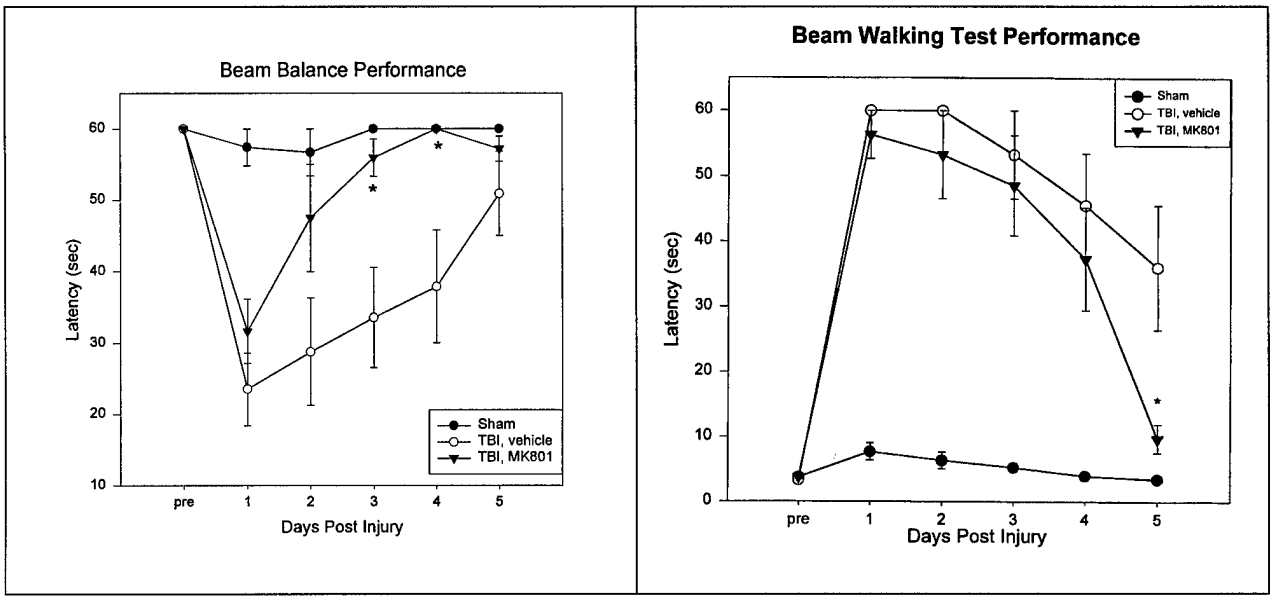


***Figure 5A-B.*** *Effect of MK-801 treatment on motor outcome after experimental TBI in rats. Mean beam balance (A) and beam walking (B) performance latencies (mean ± SEM, in sec) in rats before and on days 1-5 after CCI (4 m/s, 2.5 mm cortical deformation depth). Analysis of variance with repeated measures revealed a significant group difference. For both tests, MK-801 treated groups recovered sooner than saline treated groups (*p<0.05 vs vehicle). Data are mean ± SEM.*

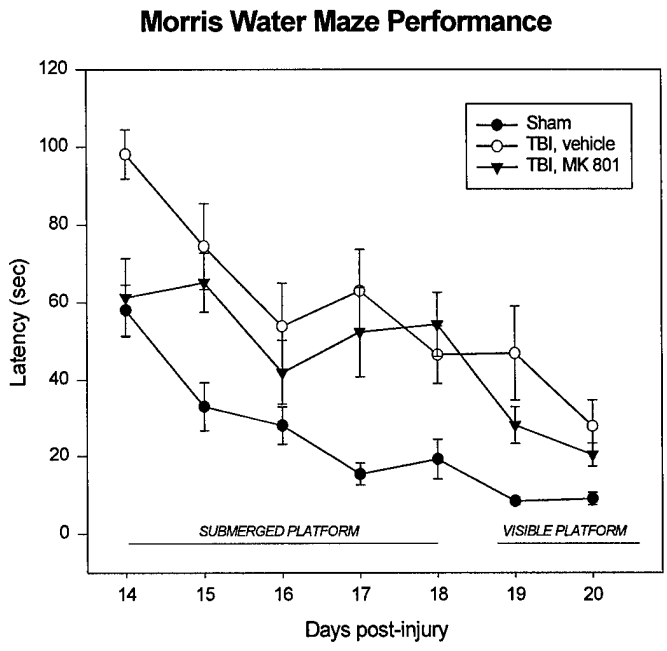


***Figure 6.*** *Effect of MK-801 on cognitive outcome after experimental TBI in rats. MWM performance latency to find a hidden platform (mean ± SEM, in sec) by rats on days 14-20 after CCI is depicted. There was no significant effect of MK-801 treatment (vs vehicle). Data are mean ± SEM*

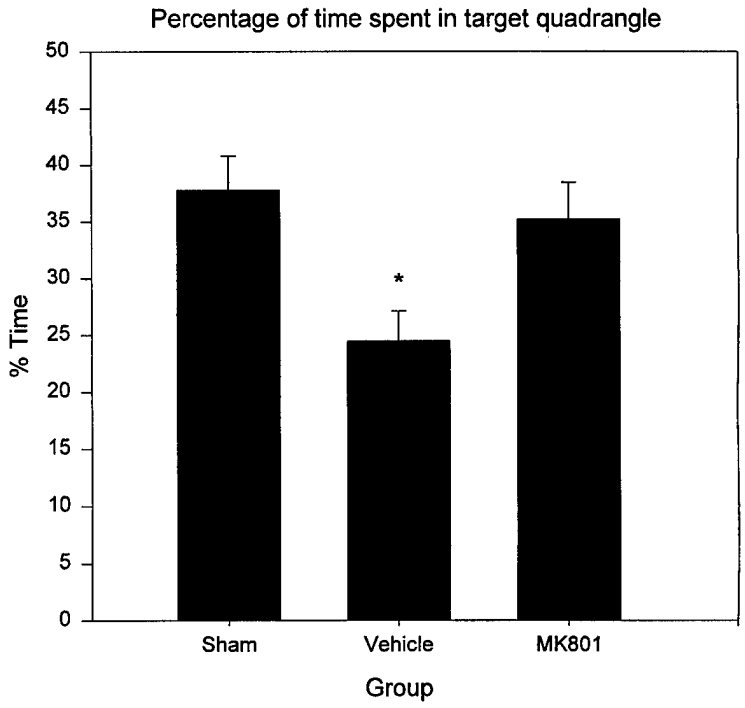


***Figure 7.*** *Effect of MK-801 on cognitive outcome after experimental TBI in rats. MWM performance probe trial (percent of time spent in target quadrant, mean ± SEM) after CCI is depicted. There was a significant beneficial effect in favor of MK-801 treatment vs vehicle treatment. Data are mean ± SEM*

Table 2. Effect of MK-801 treatment on outcome after experimental TBI in rats.

| Treatment | Lesion $mm^3$ | Lesion % non-injured hemisphere | L hemisphere | R hemisphere |
|---|---|---|---|---|
| **Vehicle** | 54.91 ± 8.35 | 12.68 ± 2.01 | 351.21 ± 17.93 | 435.92 ± 19.29 |
| **MK801** | 53.63 ± 10.00 | 13.07 ± 2.62 | 356.24 ± 23.29 | 424.61 ± 13.77 |
| **SHAM** | --- | --- | 451.29 ± 24.62 | 442/04 ± 21.62 |
| **p-value** | .92 | .90 | **0.006 *** | .81 |

All data are mean ± SEM

## Discussion

Remarkably, the NMDA antagonist MK-801 was effective in improving both motor function and some aspects of cognitive function after CCI. The motor effects were as dramatic or more dramatic than those seen with 12 hours of hypothermia. **In a separate pilot study, MK-801 was not effective when tested in our TBI plus secondary hypoxemia model, suggesting this insult may be too severe for any single therapy**. Although this specific agent is not available for clinical use, it suggests that this category of agents –targeting excitotoxicity—is a viable strategy for application with hypothermia.

**(b4)** *Comparison of the effects of TBI on functional and histological outcome after experimental TBI in rats anesthetized with isoflurane or fentanyl.*

Many, but not all, sedatives (such as barbiturates and Ketamine) target excitotoxicity. Currently, in clinical practice, fentanyl is the most commonly used emergency sedative for the

intubated patients with severe TBI. Fentanyl, has little direct anti-excitotoxic properties. Thus, we have begun to investigate how fentanyl anesthesia compared to standard isoflurane anesthesia in our model. In pilot studies, we noted that rats became markedly hypertensive and died early after TBI when anesthetized with fentanyl (but not isoflurane) in our standard TBI model. Thus, we are currently testing the use of fentanyl vs isoflurane anesthesia in our CCI model, using a slightly lesser degree of injury (2.0 mm depth of penetration rather than 2.5 mm depth—an insult with a low mortality rate in both groups). Since fentanyl is the standard of care in management of patients with TBI (in both the emergency department and the ICU), these results cold have important clinical implications if fentanyl is found to be deleterious in our model.

## (7) CONCLUSION

In our work during the second year of funding addressing portions of Technical Objective #2 and 3, we demonstrated that hypothermia plus anti-excitotoxic therapies represent an excellent potential combination therapy to test in our model of experimental TBI. In addition, we demonstrated that the combination of TBI plus a secondary insult not only results in severe deficits and large lesions after injury, but is remarkably refractory to either hypothermia or anti-excitotoxic treatment. In addition, we have begun studies suggesting that the current agent used for sedation in emergency departments and ICUs (fentanyl) may not be an optimal sedative agent. In year three we are going to first define the optimal sedative approach for field use in our TBI model (Completing Objective 2 and 3). We will then combine that approach with hypothermia in an attempt to target Objective 4 and model the best possible clinically-relevant approach for field use, both in civilian and military settings.

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J Neurosurg 88:549–556, 1998

# Augmented neuronal death in CA3 hippocampus following hyperventilation early after controlled cortical impact

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Minimizing secondary injury after severe traumatic brain injury (TBI) is the primary goal of cerebral resuscitation. For more than two decades, hyperventilation has been one of the most often used strategies in the management of TBI. Laboratory and clinical studies, however, have verified a post-TBI state of reduced cerebral perfusion that may increase the brain's vulnerability to secondary injury. In addition, it has been suggested in a clinical study that hyperventilation may worsen outcome after TBI.

*Object.* Using the controlled cortical impact model in rats, the authors tested the hypothesis that aggressive hyperventilation applied immediately after TBI would worsen functional outcome, expand the contusion, and promote neuronal death in selectively vulnerable hippocampal neurons.

*Methods.* Twenty-six intubated, mechanically ventilated, isoflurane-anesthetized male Sprague–Dawley rats were subjected to controlled cortical impact (4 m/second, 2.5-mm depth of deformation) and randomized after 10 minutes to either hyperventilation ($PaCO_2 = 20.3 \pm 0.7$ mm Hg) or normal ventilation groups ($PaCO_2 = 34.9 \pm 0.3$ mm Hg) containing 13 rats apiece and were treated for 5 hours. Beam balance and Morris water maze (MWM) performance latencies were measured in eight rats from each group on Days 1 to 5 and 7 to 11, respectively, after controlled cortical impact. The rats were killed at 14 days postinjury, and serial coronal sections of their brains were studied for contusion volume and hippocampal neuron counting (CA1, CA3) by an observer who was blinded to their treatment group.

Mortality rates were similar in both groups (two of 13 in the normal ventilation compared with three of 13 in the hyperventilation group, not significant [NS]). There were no differences between the groups in mean arterial blood pressure, brain temperature, and serum glucose concentration. There were no differences between groups in performance latencies for both beam balance and MWM or contusion volume ($27.8 \pm 5.1$ mm$^3$ compared with $27.8 \pm 3.3$ mm$^3$, NS) in the normal ventilation compared with the hyperventilation groups, respectively. In brain sections cut from the center of the contusion, hippocampal neuronal survival in the CA1 region was similar in both groups; however, hyperventilation reduced the number of surviving hippocampal CA3 neurons (29.7 cells/hpf, range 24.2–31.7 in the normal ventilation group compared with 19.9 cells/hpf, range 17–23.7 in the hyperventilation group [25th–75th percentiles]; $*p < 0.05$, Mann–Whitney rank-sum test).

*Conclusions.* Aggressive hyperventilation early after TBI augments CA3 hippocampal neuronal death; however, it did not impair functional outcome or expand the contusion. These data indicate that CA3 hippocampal neurons are selectively vulnerable to the effects of hyperventilation after TBI. Further studies delineating the mechanisms underlying these effects are needed, because the injudicious application of hyperventilation early after TBI may contribute to secondary neuronal injury.

KEY WORDS • **head injury** • **hyperventilation** • **alkalosis** • **hippocampus** • **rat**

TRAUMATIC brain injury (TBI) is often complicated by malignant intracranial hypertension,[32] which is associated with high mortality rates and has been managed using a combination of therapies including osmotherapy, diuretics, sedation, neuromuscular blockade, optimization of cerebral perfusion pressure, and hyperventilation.[6,12,32,38,51] Hyperventilation therapy has been an integral part of the clinical armamentarium in the management of severe TBI for more than 20 years:[11] this therapy rapidly reduces cerebral blood flow (CBF) and cerebral blood volume in areas of the brain with intact $CO_2$ autoregulation, providing one option in the management of TBI complicated by malignant intracranial hypertension.[1,34,42]

In recent studies, however, researchers have defined a state of reduced CBF early after TBI in humans[3,31] and animals,[5,20,25,46,56,57] particularly in the first 8 hours after TBI. Some authors have hypothesized that the brain is more

vulnerable to secondary injury during this period and that additional reduction of CBF by hyperventilation may attenuate the delivery of important energy substrates.[7,11,30,39,47,48] Yoshida and Marmarou[58] reported that hyperventilation produced relative ischemia in cat brain after fluid-percussion injury and demonstrated an increase in brain lactate and inhibition of recovery of the ratio of phosphocreatine to inorganic phosphate. Muizelaar, et al.,[40] also demonstrated a loss of brain interstitial bicarbonate buffer after sustained prophylactic hyperventilation in rabbits. It has been reported that hyperventilation after TBI in animals and humans can reduce CBF to what traditionally have been considered ischemic levels.[10,24,42] However, defining the ischemic threshold in injured tissue is problematic.[22,33] Muizelaar, et al.,[39] reported that prolonged hyperventilation after TBI in humans may worsen functional outcome, raising questions regarding the appropriate indications and timing for the optimum application of hyperventilation after TBI. Recently published guidelines for the management of severe head injury[6] state that "in the absence of intracranial hypertension, hyperventilation ($PaCO_2 \leq 35$ mm Hg) therapy should be avoided during the first 24 hours after severe TBI. . . ," although "hyperventilation therapy may be necessary for brief periods where there is acute neurologic deterioration. . . ." Consistent with these guidelines, in the setting of acute neurological deterioration, aggressive hyperventilation is used by both emergency and critical care personnel. In addition, in the initial stabilization of the brain-injured patient, aggressive hyperventilation (appropriate in the setting of impending herniation, or iatrogenic) occasionally occurs in both the prehospital and acute care settings. The specific impact of hyperventilation during this early low-flow period remains to be determined. Despite the availability of well-characterized rodent models of TBI, which reproduce the early posttraumatic reduction in CBF, the effect of aggressive hyperventilation on histopathological and functional outcome has not, to our knowledge, been investigated.

Using a rat model of focal percussive contusion, we hypothesized that aggressive hyperventilation, beginning immediately after TBI and continuing for 5 hours, would worsen functional outcome, expand the contusion, and promote neuronal death in selectively vulnerable hippocampal neurons.

## Materials and Methods

*Animals and Study Groups*

All experimental protocols used in this report were approved by the Animal Care and Use Committee of the University of Pittsburgh. Twenty-six virus-free Sprague–Dawley rats weighing 346 ± 5 g were studied. Food and water were continuously available in their home cages. After TBI the rats were randomly assigned to one of two groups of 13 animals, one receiving normal ventilation ($PaCO_2$ = 30–40 mm Hg) and one receiving hyperventilation ($PaCO_2$ = 15–25 mm Hg).

*Surgery and Brain Trauma Model*

Anesthesia was induced using 4% isoflurane in $N_2O/O_2$ (2:1). The rats were endotracheally intubated and mechanically ventilated. The isoflurane concentration was reduced to 2% followed by sterile surgical placement of a femoral arterial catheter for continuous mean arterial blood pressure (MABP) and arterial blood gas monitoring. Intramuscular injections of penicillin (100,000 U) and gentamicin (10 mg/kg) were given to minimize the risk of infection. Pancuronium bromide was administered at dosages of 0.1 mg/kg/hour via the arterial line to control ventilation. The rats' core temperature was monitored using a rectal probe.

After stereotactically guided head positioning, an incision was made and the scalp was retracted, exposing the left parietal bone. A craniotomy was made using a high-speed dental drill aided by a binocular operating microscope. A burr hole was made 5 mm anterior and 2 mm lateral to the bregma in the left side of the skull and a temperature probe (0.009-in outer diameter) was inserted through the burr hole and 2 mm into the left parietal cortex. The bone flap was left in place and the isoflurane was reduced to 1% followed by a 30-minute equilibration period. The brain temperature was maintained at 37 ± 0.5°C. Normal arterial blood gas levels were achieved in all rats and $PaO_2$ was maintained at greater than 70 mm Hg.

The TBIs were produced using a controlled cortical impact device as recently described[9,25] with minor modifications. Fifteen minutes before controlled cortical impact, an arterial blood sample was obtained for measurement of arterial blood gas levels, glucose concentration, and hematocrit. The bone flap was then removed and a vertical controlled cortical impact (4 m/second impactor velocity, 2.5-mm deformation depth) was delivered onto the exposed dura overlying the left parietal cortex. The bone flap was replaced and sealed with dental cement and the scalp was sutured.

*Study Design*

The study protocol was designed to mimic the aggressive use of hyperventilation (as opposed to normal ventilation) in the immediate posttrauma period in the prehospital as well as early hospital setting. Ten minutes after controlled cortical impact, rats were randomized to either the normal ventilation group (13 animals, $PaCO_2$ range 30–40 mm Hg) or the hyperventilation group (13 animals, $PaCO_2$ range 15–25 mm Hg). The ventilator was adjusted to maintain normocarbia or hypocarbia for 5 hours after controlled cortical impact. Arterial blood gas readings were obtained at 30 minutes post–controlled cortical impact, then hourly. The MABP was recorded every 30 minutes after controlled cortical impact. Brain and rectal temperatures were recorded every 15 minutes.

At 5 hours after controlled cortical impact, anesthesia was discontinued. Temperature probes and the femoral artery catheter were removed and the rat was weaned from mechanical ventilation in the course of 1 hour and underwent extubation. The time to extubation was recorded. After extubation, supplemental $O_2$ was administered for 30 minutes. When it had fully recovered, the rat was returned to its cage with full access to food and water.

*Functional Outcome and Behavior Assessment*

*Beam Balance.* Vestibulomotor function was tested using the beam balance test[14] in eight rats from each group. One hour before surgery, the rat was placed lengthwise on a 1.5-cm-wide beam suspended above the ground. The time the rat remained on the beam was recorded (up to 60 seconds). The rat was then removed from the beam and the procedure was repeated. Rats were considered trained when they remained on the beam for three consecutive periods of 60 seconds. Beam balance tests were also performed daily on Days 1 to 5 postinjury. Three trials were recorded and averaged each day for each rat.

*Morris Water Maze.* Cognitive function was tested in the same eight rats from each group using a standard variation of the Morris water maze (MWM) paradigm.[15,35] A pool 180 cm in diameter and 60 cm deep was painted black and filled with water to a depth of 28 cm. A clear Plexiglas platform 10 cm in diameter and 26 cm high (2 cm below the water surface) was used as the hidden goal platform. The pool was located in a 2.5 × 2.5-m room with numerous extra-maze cues (for example, posters, pipes, bookcase) that remained constant throughout the experiment. Testing started 7 days after controlled cortical impact to avoid confounding effects of motor deficits. The rats underwent four trials per day for 5 consecutive days to assess spatial memory performance. The rats started each trial once from each of the four possible start locations

TABLE 1

*Physiological values in two groups of rats treated with hyperventilation or normal ventilation after TBI**

| Value | Normal Ventilation | | Hyperventilation | |
|---|---|---|---|---|
| | Baseline | Postrandomization | Baseline | Postrandomization |
| pH | 7.39 ± 0.01 | 7.37 ± 0.01 | 7.38 ± 0.01 | 7.53 ± 0.01† |
| $PaCO_2$ (mm Hg) | 36.7 ± 1.1 | 34.9 ± 0.3 | 37.2 ± 0.9 | 20.3 ± 0.7† |
| $PaO_2$ (mm Hg) | 165 ± 6 | 167 ± 4 | 168 ± 4 | 180 ± 3† |
| base deficit (mmol/L) | 2.7 ± 3.4 | 4.2 ± 0.7 | –0.6 ± 0.9 | 4.8 ± 0.6 |
| serum glucose (mg%) | 189 ± 9 | 174 ± 6 | 158 ± 10 | 152 ± 9 |
| hct (%) | 36 ± 2.3 | 35 ± 0.6 | 32.3 ± 1.5 | 35 ± 0.6 |
| time to extubate (min) | NA | 28 ± 6 | NA | 29 ± 5 |
| brain temperature (°C) | 36.7 ± 0.1 | 37 ± 0 | 36.6 ± 0.1 | 37 ± 0 |
| rectal temperature (°C) | 36.5 ± 0.6 | 37 ± 0 | 37.1 ± 0.1 | 37.1 ± 0.1 |
| MABP (mm Hg) | 129 ± 4 | 123 ± 4 | 129 ± 8 | 128 ± 3 |

* All values are expressed as mean ± SEM. Abbreviations: hct = hematocrit; NA = not applicable.
† $p < 0.05$ at 30 minutes postrandomization compared with baseline.

(north, south, east, and west); the order of the starting location was randomized. The goal platform was positioned 45 cm from the outside wall and was placed in either the northeast, southeast, southwest, or northwest quadrant of the maze. The location of the platform was kept constant for each rat. Rats were manually placed in the pool facing the wall and were given a maximum of 120 seconds to find the hidden platform. If the rats failed to find the platform within 120 seconds, they were placed there by the researcher. All rats were allowed to remain on the platform for 30 seconds before being placed in a heated incubator between trials. There was a 4-minute intertrial interval. All data were recorded by means of a video tracking system.

*Histopathological Studies*

At 14 days after controlled cortical impact (after completion of all of the functional outcome testing), the rats were anesthetized with 5% isoflurane and killed by perfusion fixation using 10% buffered formalin. Their brains were removed and postfixed at 4°C for a minimum of 1 week, and then cryoprotected in sucrose and cut with a cryotome into 10-μ coronal sections at 1-mm increments from the occipital to the frontal lobe and stained with Cresyl violet.

*Contusion Volume.* We used a computerized image analysis system to outline the margin of the contusion and the sectional area of the contusion at each 1-mm increment was calculated by an observer (M.L.F.) who was blinded to the treatment group. Contusion volume in each rat was calculated as the sum of these sections.

*Hippocampal Cell Counting.* Neuronal loss in hippocampal regions CA1 and CA3 pyramidal layers was quantified.[8] A coronal section cut from the dorsal hippocampus underlying the area of contusion, approximately 2.6 mm posterior to the bregma, was used for analysis in each rat. The regions were visualized at × 100 magnification, then localized and counted at × 400 by an observer (R.S.B.C.) blinded to treatment group. Only complete cells with a clearly defined body and nucleus were counted. Surviving pyramidal CA1 and CA3 hippocampal neurons were counted in six separate × 400 fields for each region in both hemispheres. Sections were excluded if the boundary of the contusion extended into the pyramidal layers of the hippocampus or if fixation artifacts precluded accurate counting. Data are reported as the average number of surviving neurons per high-power field for the CA1 and CA3 hippocampal regions in both the ipsilateral and contralateral hemispheres.

*Statistical Analysis*

Survival was compared between groups using Fisher's exact test. Between group comparisons of physiological parameters, beam balance, and MWM latencies were made using one- or two-way analysis of variance (ANOVA) for repeated measures where appropriate and post-hoc tests with appropriate correction for multiple comparisons. Contusion volume was normally distributed and was compared between groups using Student's t-test. Hippocampal neuronal survival in CA1 and CA3 was not normally distributed and was compared between groups using the Mann–Whitney rank-sum test. Significance was defined at a probability level of less than 0.05.

*Sources of Supplies and Equipment*

Pancuronium bromide and gentamicin were purchased from Elkins-Sinn, Cherry Hill, NJ, and penicillin was acquired from Upjohn, Kalamazoo, MI. The stereotactic head positioning system was obtained from David Kopf, Tjunga, CA. The temperature probe was purchased from Physitemp Corp., Clifton, NJ. The video tracking system (Poly-Trak) was acquired from San Diego Instrument, Inc., San Diego, CA, and the image analysis system (MCID) was from Imaging Research, St. Catherines, Ontario, Canada.

## Results

*Physiological Parameters*

Baseline and 30-minute postrandomization physiological data are presented for all measured parameters in Table 1. After randomization, there was a marked increase in pH and decrease in $PaCO_2$ in the hyperventilation group (compared with baseline, $p < 0.05$). Hyperventilation was also associated with a small increase (12 mm Hg) in $PaO_2$ compared with baseline ($p < 0.05$). This difference was attributable to the increased minute ventilation and mean airway pressure in the hyperventilation group. At no time were any of the rats hypoxemic ($PaO_2 < 100$ mm Hg). The entire time course of $PaCO_2$, arterial pH, MABP, and brain temperature after TBI is given for both groups in Fig. 1. The $PaCO_2$ and pH levels differed between groups at all time points after randomization ($p < 0.05$). The MABP and brain temperature were similar in both groups.

Five of 26 rats died during the 14-day study, with all deaths occurring on the day of injury. Two rats remained unresponsive postinjury and were unable to demonstrate any spontaneous respiratory effort for 1 hour after discontinuation of anesthesia and were therefore killed. Three rats developed pulmonary edema and/or respiratory distress and died soon after extubation. There were no differences in mortality between groups (two of 13 in the normal ventilation group compared with three of 13 in the hyperventilation group). There were no differences between groups in time to extubation (Table 1).

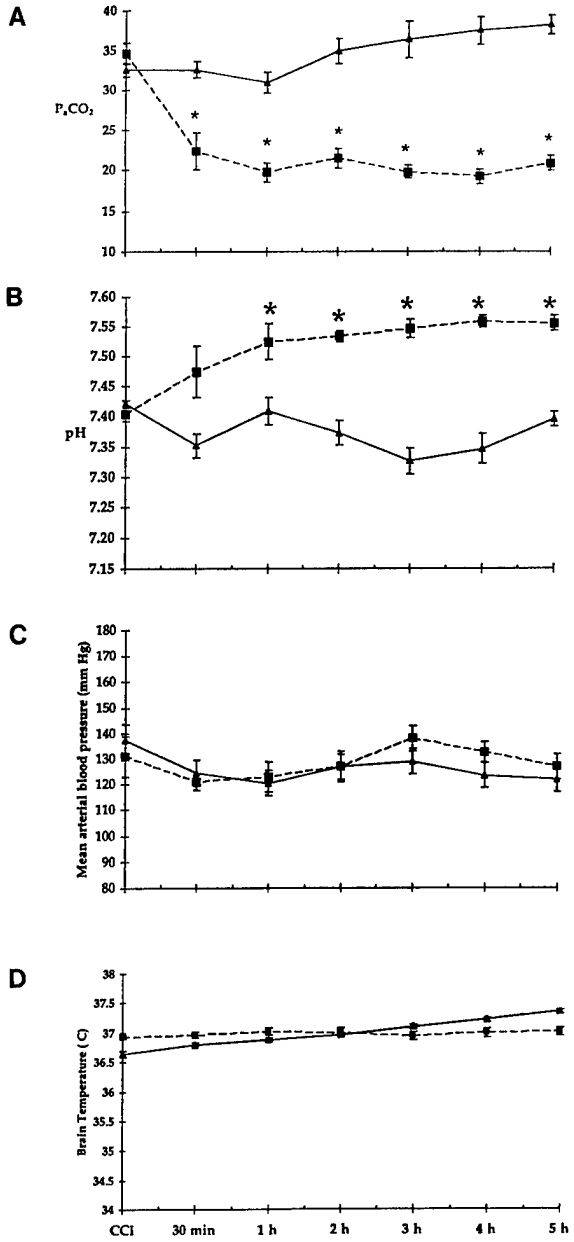


FIG. 1. Graphs showing time course of (A) $PaCO_2$ (mm Hg), (B) arterial pH, (C) MABP (mm Hg), and (D) brain temperature (°C) in all rats treated with either normal ventilation (*triangles* w/ *solid line*, 13 animals) or hyperventilation (*squares* w/ *broken line*, 13 animals) after controlled cortical impact. *$p < 0.05$ for normal ventilation compared with hyperventilation. Data are expressed as the mean ± standard error of the mean (SEM).

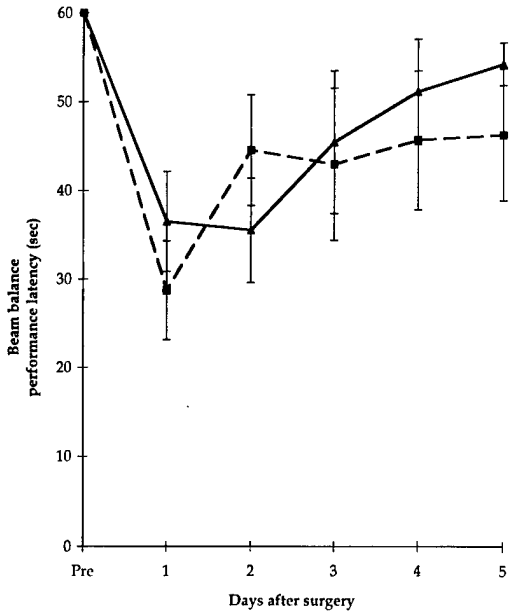


FIG. 2. Graph showing mean beam balance performance latencies (mean ± SEM, in seconds) in rats before and on Days 1 to 5 after controlled cortical impact (4 m/second, 2.5-mm cortical deformation depth). Repeated-measures ANOVA revealed no difference in duration of balance maintained between the two groups (*triangles* = normal ventilation [eight rats]; *squares* = hyperventilation [eight rats]).

### *Functional Outcome Assessment*

*Beam Balance.* There was no difference between groups in motor performance latencies over time ($F_{1,15} = 0.17$, $p < 0.69$, Fig. 2). Maximum impairment of performance occurred on Days 1 or 2 in both groups, and eventually returned to baseline. Beam balance performance did not differ significantly between normal ventilation and hyperventilation groups.

*Morris Water Maze.* There was no difference between normal ventilation and hyperventilation groups in the time needed to find the hidden platform in the MWM test ($F_{1,15} = 0.50$, $p < 0.50$, Fig. 3). In addition, there was a statistically nonsignificant tendency ($t_{13} = 1.77$, $p < 0.065$) for the rats in the hyperventilation group to swim slower than the rats in the normal ventilation group (30.8 ± 1.0 compared with 35.4 ± 2.1 cm/second).

### *Histopathological Studies*

*Contusion Volume.* At the injury level selected for this study, the contusion was generally restricted to the parietal cortex beneath the impact site. Contusion volume in both groups is shown in Fig. 4. There was no difference between groups (27.8 ± 5.1 $mm^3$ in the normal ventilation group compared with 27.8 ± 3.3 $mm^3$ in the hyperventilation group) in this outcome parameter.

*Hippocampal Cell Counting.* Figure 5 shows the number of surviving neurons/hpf in the CA1 and CA3 regions of the dorsal hippocampus ipsilateral to the contusion. There were no differences in the number of surviving CA1 hippocampal neurons between groups after controlled cortical impact. There was, however, a further reduction in the number of surviving CA3 neurons in the hyperventilation group after controlled cortical impact compared with the normal ventilation group (normal ventilation 29.7, range 24.2–31.7 neurons/hpf, compared with hyperventilation 19.9, range 17–23.7 neurons/hpf; median [25th–75th percentiles], $p < 0.05$). Neuronal cell counts in the CA1 and CA3 regions of the hemisphere contralateral to the contusion did not differ in either the normal ventilation or hyperventilation groups (CA1 counts = 55.3, range 52.1–59

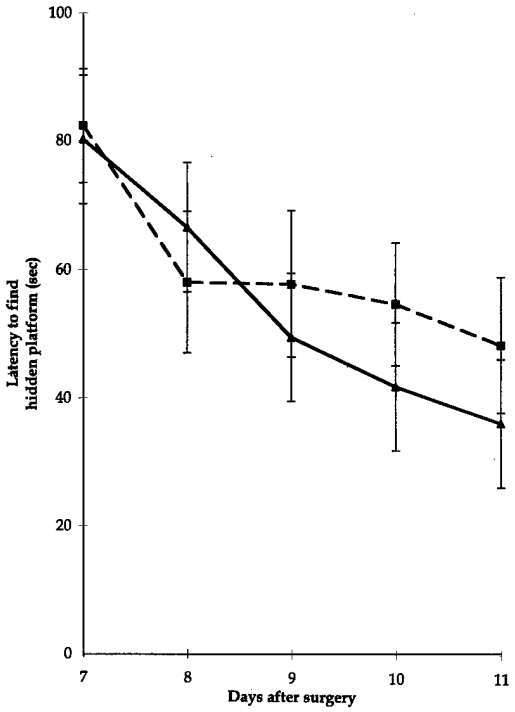


FIG. 3. Graph showing MWM performance latency to find a hidden platform (mean ± SEM, in seconds) by rats on Days 7 to 11 after controlled cortical impact. There was no difference between groups (*triangles* = normal ventilation [eight animals]; *squares* = hyperventilation [eight animals]) when performances were compared using ANOVA with repeated measures.

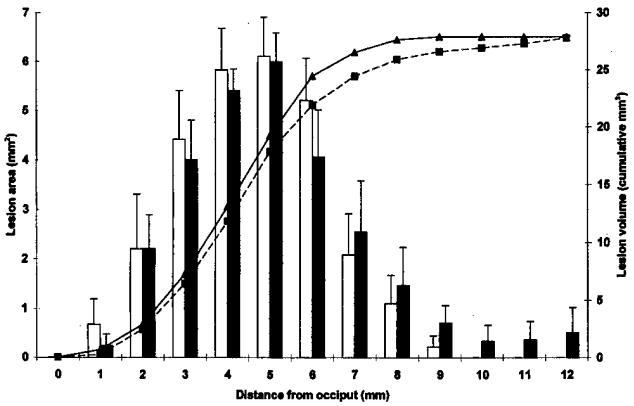


FIG. 4. Bar graph depicting mean lesion area (*left y-axis*, $mm^2$) compared with distance from occiput (mm) measured 14 days after controlled cortical impact (*open bars*, normal ventilation [11 rats]; *closed bars*, hyperventilation [10 rats]). Contusion volume ($mm^3$) was calculated as the sum of these areas in each group and is depicted as the cumulative volume *(right y-axis)* in the normal ventilation *(triangles)* and hyperventilation *(squares)* groups. There was no difference between groups in contusion volume (normal ventilation, 27.8 ± 5.1 $mm^3$ compared with hyperventilation, 27.8 ± 3.1 $mm^3$, mean ± SEM).

[normal ventilation] and 57.3, range 51.3–59 [hyperventilation]; CA3 = 40, range 36.6–41.2 [normal ventilation] and 38, range 33–41.7 [hyperventilation]).

## Discussion

In a model of controlled cortical impact–induced focal contusion in rats, aggressive hyperventilation for 5 hours after TBI augments neuronal death in the CA3 region of the hippocampus ipsilateral to the contusion. However, hyperventilation did not worsen motor function or cognitive outcome, as assessed using standard beam balance and MWM paradigms, respectively, and did not increase contusion volume.

Hippocampal CA3 neurons are selectively vulnerable to delayed neuronal death after TBI.[2,8,19,49,52,53] Theories about the mechanisms underlying this process remain speculative. Potential mechanisms include ischemia, TBI-induced excitotoxicity, apoptosis, and inflammation.[8,19,49]

Yamakami and McIntosh[56,57] reported reduced CBF as early as 15 and 30 minutes after TBI. Using a piglet model of TBI, Pfenninger, et al.,[46] reported CBF reduction as early as 5 minutes post-TBI. Some flow levels were in the range consistent with ischemia. We have previously demonstrated that the hippocampus and cortex ipsilateral to the impact show marked flow reduction (at least 60%) at 2 hours after TBI in the controlled cortical impact model.[25] Cerebral blood flow approaches ischemic levels in the core of the contusion at 2 hours postinjury. Although we have not evaluated the reactivity status of the cerebral circulation to changes in $PaCO_2$ at 2 hours after TBI in this model, we have reported that $CO_2$ reactivity is impaired, although still present (62–71% of baseline) in and around the contusion at 24 hours after controlled cortical impact in rats.[16]

Hyperventilation rapidly reduces cerebral blood volume and intracranial pressure (ICP).[11] In some studies, this intervention has been associated with CBF values consistent with ischemia or brain tissue hypoxia.[10,11,42,48] After global cerebral ischemia in dogs, hyperventilation did not increase neuronal death;[55] however, the brains were assessed at 8 hours after reperfusion, and neuronal death may be delayed. Although ischemia may be considered a contributing mechanism in the observed augmented neuronal death, ischemia alone is an inadequate explanation for our findings in light of the preservation of CA1 neurons. Although CA1 neurons are known to be selectively vulnerable to ischemic injury,[23] they were not affected by hyperventilation in this paradigm. Furthermore, in our model, CA1 neurons are more proximal to the point of impact in the cortex compared with CA3 neurons. The lack of CA1 neuronal death in light of ischemic and (presumed) anatomical vulnerability weighs against ischemia and primary injury as putative mechanisms of neuronal death in the hippocampus in this model. One limitation in this study is that neuronal counting using traditional histological methods may underestimate cell loss because of a loss of hippocampal volume.[52] We did not use stereological methods in this study. However, CA1 neuronal counts did not differ between groups and were equivalent to those observed in sham-injured animals studied in our laboratory in prior published[8] and unpublished work. In addition, comparisons were only made between injured groups within this study.

Hyperventilation produces cerebral vasoconstriction

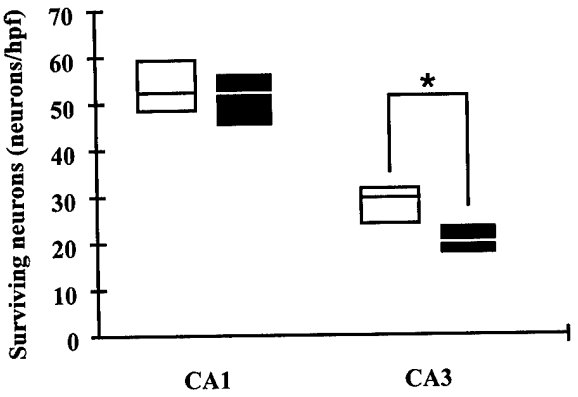


FIG. 5. Box plots representing the number of surviving CA1 and CA3 hippocampal neurons in coronal brain sections cut from the center of the lesion in the hemisphere ipsilateral to the contusion. Cells were counted 14 days postinjury. The median line is placed within the shaded 25th to 75th percentile range. There was a reduction in the number of surviving CA3 hippocampal neurons after injury in normal ventilation *(open boxes)* compared with hyperventilation *(solid boxes)* groups (29.7 cells/hpf, range 24.2–31.7 compared with 19.9 cells/hpf, range 17–23.7). *$p < 0.05$, Mann–Whitney rank-sum test.

and alkalosis.[40] Alkalosis exacerbates *N*-methyl-D-aspartate receptor–mediated neurotoxicity.[17,18,21,43] As a result of aggressive hyperventilation, the rats in our study were quite alkalotic as indicated by arterial pH measurements. Although we did not measure brain pH, a decrease in $PaCO_2$ immediately reduces brain interstitial pH.[40] Although alkalosis appears to have deleterious effects on neurons, acidosis has been shown to have both beneficial and detrimental effects. Giffard, et al.,[17] and Takadera, et al.,[54] reported a neuroprotective effect of acidosis via an attenuation of the *N*-methyl-D-aspartate receptor activation in vitro. Rosner and Becker[50] reported a deleterious effect of tissue acidosis after experimental TBI in cats. The spatial distribution of brain pH around the contusion and in the hippocampus has not been determined for either normal ventilation or hyperventilation conditions in our model.

Finally, the potential effects of hyperventilation on other mechanisms such as posttraumatic seizures or axonal injury may contribute to the enhanced vulnerability of CA3 neurons. The lateralization of the deleterious effects also raises the possibility that spreading wave depression may be a component of the neurotoxic milieu after TBI in this model of focal contusion.[20] It could also be the case that the combined effect of alkalosis and further flow reduction by hyperventilation is deleterious in regions vulnerable to excitotoxicity such as CA3. Early, aggressive, or prophylactic hyperventilation, therefore, in the context of reduced CBF, may potentiate excitotoxic mechanisms and augment neuronal death.

Aggressive hyperventilation in the early low-flow period did not worsen functional outcome or expand the contusion, failing to support a significant portion of our initial hypothesis. Ultimate contusion size, in controlled cortical impact or other models of focal contusion, is relatively refractory to manipulation by a variety of interventions;[4,8] however, application of hypothermia, particularly prior to injury, reduces contusion volume resulting from controlled cortical impact and lateral fluid-percussion injury.[13,44] Although we chose rather aggressive hyperventilation in an attempt to produce a maximum effect, we did not test the effect of hyperventilation on a milder contusion, which may be more manipulable to secondary insults. The contusion penumbra has not been clearly defined in either of the standard rodent TBI models (controlled cortical impact or fluid–percussion) for any level of injury. It is possible that selectively vulnerable CA3 hippocampal neurons are the only potential target for a deleterious effect of hyperventilation in our model. However, the effect of hyperventilation on the survival of neurons in the dentate gyrus or hilus (all vulnerable to TBI)[9,29] was not assessed.

Hippocampal damage and memory deficits are common after TBI in humans.[26,28] This study did not reveal any added effect of hyperventilation on functional outcome deficits as measured by beam balance and MWM latencies. A number of factors may have contributed to this. Our sample size may have limited statistical power; however, this sample size was adequate to detect the exacerbation of functional deficits by the addition of 30 minutes of moderate hypoxemia ($PaO_2$ 40 mm Hg) in our model.[8] Second, the cognitive deficits in this model are modest compared with those detailed in previous reports.[15] Bilateral hippocampal damage may be necessary to create more marked functional deficits.[36,37] In addition, CA3 damage may not mediate post-TBI memory deficits, as manifested in MWM test results. Finally, the specific functional outcome paradigm may not have the necessary sensitivity to detect subtle functional deficits. For example, more demanding MWM paradigms have been used by other investigators.[27,52] However, in support of the testing strategy used, our hypothesis was that hyperventilation would worsen functional deficits.

This study does not completely address the uncommon situation in which, soon after severe head injury, marked intracranial hypertension is observed. Hyperventilation may in fact be life saving in its ability to impede herniation. Similarly, we did not measure ICP or titrate ventilation to control cerebral perfusion pressure, and we evaluated only one level of hyperventilation and injury severity. We did not attempt to model the clinical scenario of optimum titration of ventilation when ICP is increased. In the clinical setting, some investigators have demonstrated a wide variety of beneficial effects of hyperventilation under those conditions, such as homogenization of CBF, normalization of cerebral glucose uptake, and improvement in autoregulation.[12,41,42] Rather, we chose the worst-case scenario, aggressive hyperventilation during the early posttrauma period when flow is already low and excitotoxicity is peaking.[45] However, our study does show that hyperventilation is associated with a tangible risk to vulnerable neurons in the controlled cortical impact model. To our knowledge, this is the first in vivo study demonstrating that hyperventilation can augment neuronal injury after TBI, suggesting that there is indeed a tradeoff associated with this intervention.

## Conclusions

We have demonstrated that aggressive, early hyperven-

tilation after TBI augments neuronal death in CA3 hippocampus. The further reduction of CBF with hyperventilation during the low CBF state immediately after severe TBI, coupled with alkalosis, may increase the vulnerability of selected neurons to traumatic injury. Further studies are needed to delineate the relative contributions of these mechanisms to the observed effects. The results of this study reinforce that meticulous attention is necessary to prevent secondary injury after TBI, and a risk in the use of hyperventilation is demonstrated.

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## 5

CHANGES IN MITOCHONDRIAL MEMBRANE POTENTIAL IN STRETCH-INJURED ASTROCYTES AND NEURONS. S.M. Ahmed*, B.A. Rzigalinski and E.F. Ellis, Dept. of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA.

The dynamics of energy failure in traumatically injured astrocytes and neurons are unclear. In order to better understand mitochondrial function and cell energetics following trauma we utilized the fluorescent dye Rhodamine 123, which is normally sequestered in mitochondria where its fluorescence is quenched. When mitochondrial membrane potential (MMP) decreases, such as with mitochondrial poisons, the dye moves to the cytoplasm and fluorescence is increased. Pure neuronal or astrocytic cultures were subjected to mild (5.7 mm), moderate (6.5 mm) or severe (7.5 mm) stretch-induced injury and the change in MMP measured. There were no significant changes in MMP in mildly to moderately injured neurons at 15 min, 24 or 48 hr post-injury. However severely injured neurons displayed an immediate 33% decrease in MMP that persisted to 48 hr. In contrast, mild and moderate astrocyte injury caused a dramatic, 39-52% drop in MMP at 15 min, with MMP returning to normal by 24 hr. Our results indicate that direct trauma-induced alterations in cell energetics vary greatly in neurons and astrocytes. We suggest that in vivo the deficit induced in astrocytes may alter astrocyte function, which in turn may produce dramatic effects on neuronal function. Supported by NS-27214 and NS-07288.

## 6

HYPERTHERMIA ADVERSELY AFFECTS OUTCOME AFTER MODERATE HEAD INJURY. Philipp R. Aldana[1*], J. Marquez[1], D. S. Petrin[1], D. Johns[1], W. D. Dietrich[2], P. A. Villanueva[1]. Department of Neurological Surgery[1], Neurotrauma Research Center[2], University of Miami School of Medicine, Miami, Florida, 33101, USA.

Hypothermia has been shown to have beneficial effects after traumatic brain injury (TBI) in both human and animal studies. Conversely, hyperthermia after TBI has been shown to have deleterious effects in animals. No studies have addressed the effects of hyperthermia after moderate head injury in humans.

104 patients admitted with a Glasgow Coma Score 9-12 due to blunt head trauma were studied. Demographics, comorbid factors and characteristics of the hyperthermic episodes (>38.6°C) were examined. The number of patients either dead, in a vegetative state or severely disabled during discharge was significantly larger for the hyperthermic group vs. the normothermic group (42.4% vs. 17.5%, respectively). A significantly larger percentage of the normothermic group had a good outcome compared to the hyperthermic group (50% vs. 20.3%, respectively). Among the hyperthermic patients, those with associated infections had significantly worse outcomes and a higher frequency of hyperthermic episodes than those without infections. We conclude that hyperthermia in the face of an associated infection may adversely affect the outcome of patients with moderate head injury. We advocate maintenance of at least normothermic conditions if moderate hypothermia cannot be achieved and treatment of any underlying infection after TBI.

## 7

**EVIDENCE FOR APOPTOTIC CELL DEATH FOLLOWING SUBDURAL HEMATOMA IN RATS.** B. Alessandri*, X. Di, H. Chen, R. Bullock. Div. of Neurosurgery, Medical College of Virginia, Richmond, VA, 23298, USA

Subdural hematoma (SDH) is a common and dangerous secondary event following traumatic brain injury. The mechanisms leading to neuronal death, even after SDH removal, are not fully understood. A mechanism which might contribute to cell death is apoptotic cell death (ACD), which has been shown to be involved in the development of traumatic and ischemic brain damage.

The hematoma was produced by subdural injection of 250μL of autologous venous blood in Halothane anesthetized rats. Animals were allowed to survive 1 (n=3), 2 (n=3), 4 (n=3) or 7 days (n=4) after injection of SDH. Brain sections were stained by a commercially available apoptosis detection kit (FragEL™) for apoptotic cells (visualized by dianinobenzidine, DAB; counterstained by hemotoxylin). Brain sections were examined light microscopically and DAB-positive cells were counted in both hemispheres in cortical, subcortical and hippocampal areas.

The DBA-pos. cell counts were 2.3±2, 54.5±8, 13.7±8, and 12.8±3 at 1, 2, 4 and 7 days after SDH, respectively All apoptotic cells were within the cortex, within and in the border zone of the SDH lesion. There were no DAB-pos. cells in the contralateral side. The number of DAB-pos. cells was highly correlated with the lesion area ($r^2$=0.689, p<0.001).

The results indicate that ACD occurs following SDH, and is maximally seen at 2 days. DAB-pos. cells were only found within or in the border zone of the lesion. The correlation of ACD and lesion area underlines the importance of this type of cell death in SDH. The contribution of ACD to SDH-induced brain damage and its relevance for therapy needs further study.

## 8

**VERTICAL VERSUS ANGLED CONTROLLED CORTICAL IMPACT IN RATS.** H.L. Alexander*, C.L. Robertson, C.E. Dixon, R.S.B. Clark, S.H. Graham, P.J. Safar, P.M. Kochanek. Safar Center for Resuscitation Research, Univ. of Pittsburgh, PA 15213

Although a variety of modifications of the controlled cortical impact (CCI) model exist, a comparison between the two most common variants, vertical[1] and angled impact[2], has not been performed. Rats were subjected to vertical (n = 8), angled (n = 8) or sham (n = 8) insults (4 m/s, 2.5 mm) to the left parietal cortex, using a CCI model with hypoxemia.[3] Motor (beam balance, d1-5), cognitive (Morris Water Maze, d14-21) and histologic (lesion volume, CA1 and CA3 neuron counts, d21) outcomes were studied. Motor and MWM performance were impaired, but did not differ between injury groups. Lesion volumes also did not differ (vertical = 92.2±7.2 $mm^3$, angled = 79.4±7.8, $p$ = 0.25). CA1 neuron counts were decreased ipsilateral to injury in both groups vs sham (vertical = 20.4±8.4 cells/hpf, angled = 32.7±15.8, sham = 55.5±3.9, $p < .05$). However, CA3 neuron counts were decreased ipsilateral to injury in the vertical group vs sham (23.2±8.5 vs 52.1±6.6, respectively, $p < .05$), but the angled group (32.7±15.8) was not different from sham. We conclude that the vertical and angled variants of the CCI model produce similar functional deficits; however, the vertical impact appears to produce greater local damage, particularly in CA3 neurons. *[1]J Neurotrauma 12:1015 [2]J Neurosci Methods 39:253; [3]J Neurotrauma 14:179; Support: US Army #DAMD17-97-1-7009*

## 45

CHRONIC OVEREXPRESSION OF AMYLOID PRECURSOR PROTEIN (APP) AFTER TRAUMATIC BRAIN INJURY IN RATS. J. R. Ciallella[1]*, H. Q. Yan[1], X. Ma[1], D. W. Marion[1], S. T. DeKosky[2], and C. E. Dixon[1]. Departments of [1]Neurosurgery and [2]Psychiatry, University of Pittsburgh Medical Center, Pittsburgh, PA USA.

Traumatic brain injury (TBI) and Alzheimer's disease (AD) produce cholinergic and metabolic deficits that may contribute to neurodegeneration. There is increasing evidence linking AD and TBI, including upregulation of APP in head injured patients (McKenzie et al. 1994 *NeuroRep.*6:161). To further investigate this linkage, we tested the hypothesis that controlled cortical impact (CCI) injury would produce chronic upregulation of APP protein levels at 4 weeks following injury. Our previous studies demonstrated significant changes in cholinergic proteins at this time point (Ciallella et al. 1998. *Exp. Neurol.* In Press). APP immunohistochemistry (n=3-5) and western blot (n=4) were performed on cortical and hippocampal regions from injured and sham animals. The same N-terminal antibody was used in all studies. A marked increase in cortical and hippocampal APP protein was demonstrated bilaterally by both immunohistochemistry and western blot in injured rats compared to sham controls. This demonstrates that a single TBI can lead to chronic upregulation of APP, concurrent with chronic alterations in cholinergic markers. Supported by AG05133, NINDS-T32NS07391, CDC-CCR312296, NIH-NS30313, and NIH-NS33150.

## 46

**EFFECT OF HYPOTHERMIA AFTER SEVERE TRAUMATIC BRAIN INJURY WITH SECONDARY HYPOXEMIA IN RATS.** R.S.B. Clark*, C.L. Robertson, C.E. Dixon, H.L. Alexander, S.H. Graham, P.J. Safar, P.M. Kochanek. Safar Center for Resus Res., U of Pgh, PA 15213.

Many reports have shown benefit from hypothermia (HT) in traumatic brain injury (TBI); but, its effect on TBI with secondary insult remains undefined. We hypothesized that HT would improve outcome after controlled-cortical impact (CCI) with secondary hypoxemia. Rats received severe CCI injury followed by 30 min of hypoxemia,[1] and randomized to normothermia (NT=37°C brain temp, n=19), immediate HT (IHT=32°C, after CCI, n=10), or delayed HT (DHT=32°C, after hypoxemia, n=14) for 4 h. Motor (beam balance/ walking, d1-5), cognitive (Morris Water Maze [MWM], d14-21) and histologic outcomes (lesion volume, hippocampal neuron counts, d21) were evaluated. Motor and MWM performance were impaired but did not differ between groups. Lesion volumes ($mm^3$) did not differ between groups (NT=65.3±6.9, IHT=50.2±8.2, DHT=53.7±7.9). Neuron counts (CA1, CA3) were decreased 60-70% ipsilateral to CCI, but did not differ between groups. Mortality doubled (43% vs 20-21%) in DHT vs NT or IHT ($p$ = 0.3). HT did not improve outcome after severe CCI with secondary insult. Clinical studies[2] exclude patients with secondary insults, and suggest HT is not effective after severe injury (GCS 3-4). Novel therapies may be needed in this setting. *[1]J Neurotrauma 14:179; [2]NEJM 336:540-6; Support: US Army #DAMD17-97-1-7009*

## 47

THE SUPPRESSION OF HIPPOCAMPAL NGF mRNA AFTER CEREBRAL ISHCEMIA IN RAT TREATED WITH ANTISENSE DNA TO C-*fos*. J-K.Cui[1]*, C. Y. Hsu[2], and P. K. Liu[1]. [1]Department of Neurosurgery, Baylor College of Medicine, Houston, TX 77030; [2]Department of Neurology, Washington University, St Louis, MO 63110.

The biological effects of Fos expression in the brain were examined using phosphorothioated oligodeoxy-nucleotides (s-ODNs) to c-fos, $rncfosr_{115}$. Biotinylated antisense $rncofsr_{115}$ (bio- $rncfosr_{115}$) plus lipofectin were delivered into the brain of male Long-Evans rats (225-250 gm) via intracerebroventricular infusion. The distribution of bio-$rncfosr_{115}$ was detected using antibodies against biotin. Using dot blot analysis on the recovered bio-$rncfosr_{115}$, the bio-$rncfosr_{115}$ uptake in hippocampus peaked at 29-48 hrs, and the internalized bio-$rncfosr_{115}$ was degraded within 72 hr of infusion. The s-ODN uptake in the brain was confirmed by 3'-end-labeling with digoxigenin-dUTP, using terminal transferase and anti-digoxigenin IgG-FITC. The presence of fluorescent aggregates in the brain cells near the vessel wall in animals treated with antisense $rncfosr_{115}$ + lipofectin suggests lipofectin mediated s-ODN transfer across the blood brain barrier. The uptake increased with time and with the dose delivered. The effectiveness of antisense $rncfosr_{115}$ was shown by an inhibition of ischemia-induced Fos expression, and was accomplished by an inhibition of ischemia-induced hippocampal NGF mRNA expression in the brain of animals pretreated with antisense $rncfosr_{115}$. The specificity of Fos suppression was suggested by a lack of antisense $rncfosr_{115}$ effect on the expression of NT-3 and α-actin mRNA.

## 48

LOSS OF GLIAL POTASSIUM CURRENTS AND IMPAIRMENT OF POTASSIUM HOMEOSTASIS, FOLLOWING FLUID PERCUSSION INJURY. R. D'Ambrosio*, D.O. Maris, M.S. Grady, and D. Janigro. Dept. of Neurosurgery, Univ. of Washington, Seattle, WA 98104

We compared the early effects of moderate in vivo fluid percussion injury (FPI) on the functional expression of potassium currents expressed in oligodendroglia and astrocytes from acutely isolated rat hippocampal slices. Whole cell recordings were performed from post–FPI and naïve slices of 30 d.o. rats. Cs+ (1 mM) was used to block inward potassium currents. K+-selective electrodes were employed to measure K+ accumulation in radiatum CA3. GFAP immunostaining was enhanced in CA3 24-48 hrs following FPI, while immunostaining for oligodendroglia was reduced. A significant decrease in Cs+-sensitive potassium currents was observed following lesion in both oligodendroglia and astrocytes. Cells characterized by complex electrophysiological profiles as well as those characterized by inward rectification were equally affected (-60% and –55% at –140 mV). Morphologically, complex cells visualized by biocytin staining could be classified as oligodendrocytes. Stimulation (1 Hz) of Schaffer collaterals induced K+ accumulation in radiatum CA3. Slices obtained from naïve rats always showed a recovery of extracellular K+ to basal levels within 10 seconds following stimulation (n=5). Slices obtained from post-FPI rats displayed recovery times ranging from 10 to 40 seconds (n=8). Additionally, 75% of the post-FPI slices generated multiple afterdischarges during stimulation, while only 20% of the control slices did. These results indicate that 1) post-FPI CA3 astrocytes are reactive or injured, 2) loss of Cs+-sensitive potassium current occurs in oligodendrocytes and astrocytes post-FPI, 3) neuronal-activity-induced elevation of $[K+]_{out}$ is more persistent at early time point post-FPI, 4) hyperexcitability is observed after trauma without detectable neuronal loss. We conclude that FPI may affect extracellular K+-homeostasis by impairing glial potassium currents. Supported by NIH-51614 and RO-1 NS33107.

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# NO LONG-TERM BENEFIT FROM HYPOTHERMIA AFTER SEVERE TRAUMATIC BRAIN INJURY WITH SECONDARY HYPOXEMIA IN RATS

Courtney L. Robertson, Robert Clark, C. Edward Dixon, Steven Graham, Henry Alexander, Stephen Wisniewski, Donald Marion, Peter Safar, Patrick Kochanek. Depts of Anesthesiol/CCM, Pediatrics, Neurology, Epidemiology, and Neurosurgery, Safar Center for Resuscitation Research, University of Pittsburgh, PA 15213.

**Introduction:** Many reports have shown benefit from hypothermia in traumatic brain injury (TBI); but its effect in the setting of TBI with secondary insult remains undefined. Clinical studies show an increase in morbidity and mortality after severe TBI with secondary brain insult.[1] In experimental rat models, outcomes were worse in brain injury with secondary hypoxia.[2] Recently, we characterized a model of TBI with secondary hypoxemia and reported prominent neuronal apoptosis after injury.[3,4] We hypothesized that hypothermia would improve outcome after controlled-cortical impact (CCI) with secondary hypoxemic insult in rats.

**Methods:** Rats were subjected to severe CCI injury followed by 30 min of hypoxemia ($PaO_2$=35-45 mm Hg).[3] Rats were then randomized to normothermia (NT=37°C, n=19), immediate hypothermia (IHT=32°C, after CCI, n=10), or delayed hypothermia (DHT=32°C, after hypoxemia, n=14) for 4 h. Motor (beam balance/ beam walking, d 1-5), cognitive (Morris Water Maze [MWM], d 14-21) and histologic outcome (lesion volume, hippocampal neuron counts, d21) were evaluated.

**Results:** Motor and MWM performance were impaired, but did not differ between groups. Lesion volumes did not differ significantly between groups (NT=65.3 $mm^3$ ±6.9, IHT=50.2±8.2, DHT=53.7±7.9). Hippocampal neuron counts (CA1,CA3) were decreased on the injured side, but did not differ between groups (NT-CA1=19.8±4.2 cells/hpf, NT-CA3=19.8±4.6, IHT-CA1=13.2±8.7, IHT-CA3=15.6±7.3, DHT-CA1=13.7±5.8, DHT-CA3=18.5±7.3). Mortality rate did not differ significantly between groups.

**Conclusions:** Immediate or delayed hypothermia did not improve long-term outcome after severe CCI with secondary hypoxemia in rats. The severity of the combined insult may be outside of the therapeutic window of opportunity. Clinical studies[5] have excluded patients with secondary insult, and have indicated that hypothermia is of limited efficacy in the subset of severely injured (GCS 3-4) patients. Novel therapeutic approaches or combination therapies may by necessary in this setting. **[1]*J Trauma* 34:216, [2]*J Cereb Blood Flow Metab* 7:759, [3]*J Neurotrauma* 14:179,[4]*J Neurosci* 17:9172, [5]*NEJM* 336:540;** **Support: USArmy#DAMD17-97-1-7009**

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## 101.5

EFFICACY OF CASPASE INHIBITION FOR INTRACEREBRAL HEMORRHAGE IN RATS. K.Matsushita[1], W.Meng[2], M.Yamada[1], M.A.Moskowitz[1], E.H.Lo*[2]. [1]Stroke and Neurovascular Regulation, [2]Neuroprotection Research Laboratory, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA02129, USA.

Compared with ischemia, the mechanisms that underlie neuronal damage following intracerebral hemorrhage remain relatively unexplored. Parenchymal ischemia accompanying hemorrhage is typically mild (CBF 50-75% of baseline); therefore this may favor apoptotic pathways of neuronal cell death. The aim of the present study is to characterize the spatial and temporal profile of apoptosis after hemorrhage and evaluate the therapeutic efficacy of caspase inhibition. In vitro experiments confirmed that collagenase per se was not toxic in cultured neurons. Intrastriatal hemorrhage was then produced in rats by the intracerebral injection of collagenase (0.5u in 1μL). Nissl and TUNEL staining at 24, 48 and 72 hrs post-hemorrhage demonstrated that TUNEL positive apoptotic cells were distributed more in the periphery than in the center between 24 and 48 hrs, and then declined in number at 72 hrs. Pre-treatment with the caspase inhibitor, z-VADfmk (80ng, icv), significantly reduced the number of TUNEL positive cells at 24 hrs.

These findings suggest that apoptosis is an important pathological mechanism following intracerebral hemorrhage and caspase inhibition may have a therapeutic effect.

Supported by Uehara Memorial Foundation (KM), NS10828 (MAM), NS32806 (EHL), and NS37074 (EHL).

## 101.6

INHIBITION OF INTERLEUKIN 1β CONVERTING ENZYME FAMILY PROTEASES REDUCES COLD INJURY-INDUCED BRAIN TRAUMA AND DNA FRAGMENTATION IN MICE. Y. Morita-Fujimura,[2] M. Fujimura,[2] M. Kawase,[2] K. Murakami,[2] L. Litt[1] and P. H. Chan[2]. [1]Dept. of Anesthesia, Univ. of California, San Francisco; [2]Dept. of Neurosurgery, Neurology and Neurological Sciences, Stanford Univ., School of Medicine Palo Alto, CA 94304.

The interleukin 1β converting enzyme (ICE) family, a protease family implicated in apoptosis, has been reported to be activated after brain injury such as ischemia and trauma, and its inhibitors reduce ischemic brain infarction (Hara *et al.*, 1997, Yakovlev *et al.*, 1997). We examined the effect of z-VAD.FMK, a relatively nonselective inhibitor that blocks both ICE-like and CPP32-like caspases, on cold injury-induced brain trauma in which apoptosis appears to play a role (Tominaga *et al.*, 1992). The vehicle alone or with z-VAD.FMK was intracerebroventricularly administered to mice 15 min before and 24h and 48h after cold injury. At 4h after cold injury, infarction volumes in z-VAD.FMK-treated animals were significantly smaller than infarction volumes in vehicle-treated animals, which were further decreased at 24h and 72h (0.92±1.80 $mm^3$ ; z-VAD.FMK-treated animals, 7.46±3.53 $mm^3$ ; vehicle-treated animals, mean ± S.D., n=8). The amount of apoptotic cell death was significantly decreased in z-VAD.FMK-treated animals compared with vehicle-treated animals, as shown by TUNEL staining and DNA gel electrophoresis. Although further investigation is necessary to elucidate mechanisms of ICE inhibitor effects on cold injury-induced brain trauma, these data suggest that ICE inhibitors might be of therapeutic benefit in brain trauma. The ICE family of proteases appears to contribute significantly to cold injury-induced brain trauma. Blocking ICE activity increases neuronal survival by reducing apoptosis. Supported by grants NS14543, NS25372, NS36147 and NOINS82386.

## 101.7

AXONAL PROTECTION WITH HYPOTHERMIA FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT. H. Koizumi, J.T. Povlishock*. Dept. of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

The protective effects of mild hypothermia following traumatic brain injury (TBI) have been demonstrated in multiple studies within the last decade. However, while this protection has been evaluated in relation to the preservation of neurons and/or the blunting of behavioral abnormalities, little consideration has been given to any potential protection afforded to TBI-induced axonal injury, a known feature of human TBI.

To this end, we evaluated the protective effects of mild hypothermia on axonal injury following TBI in rats. Male Sprague Dawley rats weighing 380-400 grams were subjected to experimental TBI induced by impact acceleration. These rats were also subjected to hypothermia either prior to injury or up to 1 hour postinjury, with their temporalis muscle and rectal temperature maintained at 32°C for an 1 hour period. After this 1 hour period of hypothermia, gradual controlled rewarming to normothermic levels was accomplished over a 90 minute period. Twenty-four hours later, the animals were perfused and semiserial sagittal sections of the brain were reacted for the visualization of the amyloid precursor protein (APP), a known marker of axonal injury. The density of APP/damaged axons within the corticospinal tract at the pontomedullary junction was calculated for each animal.

In all hypothermic animals, a significant reduction in APP/damaged axonal density was found. With pre-injury, immediate postinjury, and delayed hypothermia, the density of damaged axons was dramatically reduced in comparison to the non-treated controls ($p < 0.05$). These findings indicate that early as well as delayed post-traumatic hypothermia result in considerable protection of those axons injured by the traumatic episode. (supported by NS 20193)

## 101.8

DNA DAMAGE IS TEMPERATURE DEPENDENT EARLY AFTER TRAUMATIC BRAIN INJURY IN RATS M. Whalen, M. Chen*, R. Clark, K. Jin, P. Kochanek, D. Marion, S. Graham. Safar Center for Resuscitation Research and Brain Trauma Research Center, University of Pittsburgh, Pittsburgh, PA 15260

Hypothermia applied before or shortly after traumatic brain injury (TBI) attenuates while hyperthermia exacerbates neurologic damage in experimental TBI (Dietrich et al., 1996). DNA damage occurs in neurons undergoing necrosis and apoptosis after TBI (Clark et al., 1997). One mechanism by which hypothermia might mitigate neurologic injury is suppression of neuronal DNA damage. We hypothesized that neuronal DNA damage after TBI would be temperature-dependent within a clinically relevant range. Anesthetized male adult Sprague-Dawley rats were subjected to controlled cortical impact and maintained at brain temperature 32, 37, or 39°C ( ± 0.5°C; n=8/group) for 4 h. Coronal (6 μm) cryostat brain sections were then obtained through the center of the contusion. DNA damage was assessed using biotinylated dATP and the Klenow fragment of DNA polymerase I. DNA damage was quantified by light microscopy as the number of positively-labeled cells/100x field in cortex and hippocampal regions. Data are expressed as mean ± SEM. Results were analyzed by ANOVA and Student-Neuman-Keuls test. DNA damage was evident in many cells in the ipsilateral cortex, dentate, and CA3 hippocampus, but was rarely detected in CA1 or the contralateral hemisphere. DNA damage was temperature-dependent in the dentate gyrus (9.8 ± 5.0 vs 31.0 ± 8.3 and 63.6 ± 18.1)( 32°C vs 37°C and 39°C, respectively; $p < 0.05$) and CA-3 (4.1 ± 2.1 vs 13.0 ± 2.2)(32°C vs. 39°C; $p < 0.05$), but not in CA-1 or regions of the cortex adjacent to the impact site. DNA damage in regions of hippocampus vulnerable to delayed neuronal death seems to be temperature-dependent early after TBI. **One beneficial effect of hypothermia may be inhibition of DNA damage after TBI. Funding: Charles Schertz Fellowship Grant from the Univ. Pitt. Dept. Anesthesiology/CCM, NS30318, KO8NS01946**

## 101.9

ALTERED EXPRESSION OF ENDOTHELIN-1 AND THE ENDOTHELIN B RECEPTOR SUBTYPE (ETB) AFTER SPINAL CORD INJURY. J. A. Ellison, A. E. M. Mautes, H. Minehart, R. Willette, and L. J. Noble*. Depts. of Neurosurgery, University of California at San Francisco and Saarland University Medical School, Homburg/Saar, Germany, and Dept. of Cardiovascular Pharmacology, SmithKline Beecham Pharmaceuticals.

Glial activation is a prominent feature of the injured spinal cord. There is increasing evidence that ET-1 may participate in glial activation via the ETB receptor. In this study, we begin to address this putative role of ET-1 in the contused spinal cord of the rat.

At 3 hours to 3 weeks after a moderate spinal cord injury or after sham surgery, a 3 cm length of cord, centered over the impact or sham surgery, was removed and divided into proximal, injury, and distal segments. Sections were prepared for immunolocalization of ET-1 and in situ hybridization analysis of ETB mRNA expression. Levels of mRNA expression were quantitated by optical density analysis of the xray film exposed to slides hybridized with the ETB probe. The data were analyzed using Kruskal-Wallis, followed by Mann-Whitney U.

There is enhanced immunoexpression of ET-1 at all time points and a significant increase in ETB mRNA signal along the axis of the injured cord at 1 to 3 weeks post injury as compared to sham surgery. ET-1 is localized in reactive glia, bordering central and dorsal column cavities, and macrophage-like cells. There is pronounced ETB mRNA in similar phenotypes in the lesion and bordering the penumbra of the injury from 1 to 3 weeks post injury.

The enhanced expression of ET-1 and ETB mRNA in glial and macrophage phenotypes suggest that local ET-1 may influence both glial reactivity and macrophages. Supported by NS23324.

## 101.10

THE ROLE OF CALPAIN-MEDIATED SPECTRIN PROTEOLYSIS (CMSP) IN TRAUMATICALLY INDUCED AXONAL INJURY (AI). A. Büki[1], J.T. Povlishock[1], R. Siman[2] and C.W. Christman[1]* [1]Dept. of Anatomy, Medical College of Virginia; Virginia Commonwealth University, Richmond, VA 23298-0709, [2]Cephalon, Inc., West Chester, PA 19380

Traumatic brain injury (TBI) has long been associated with generation of AI. Such axons are not mechanically severed at impact, instead showing progressive changes that lead to axonal disconnection. In severely injured axons, it has been shown that the axolemma is perturbed, suggesting the influx of $Ca^{2+}$ and the unleashing of $Ca^{2+}$-mediated overt proteolytic degradation. Experimental studies, however, have failed to confirm this assumption, suggesting that alterations in axonal permeability trigger more discrete and evolving cytoskeletal changes.

To explore the role of $Ca^{2+}$-induced proteolysis in AI, this study was undertaken in an animal model of TBI coupled with antibodies targeting both CMSP and focal neurofilament compaction (NFC). Rats were subjected to impact acceleration TBI and allowed to survive for 15 min to 2 h, when the brains were prepared for the visualization of double label reaction products related to the presence of CMSP and NFC. Using LM and EM, these strategies revealed that TBI consistently evoked focal CMSP immunoreactivity (IR). This focal IR was also correlated with concomitant change in the underlying cytoskeleton reflected in NFC. These changes were seen at 15 min postinjury and continued over the entire 2 h observation period. We confirmed these changes at the EM level. At 15 min post injury, IR associated with CMSP was confined primarily to the subaxolemmal network. With increasing survival, its distribution became more widespread moving from the subaxolemmal compartment to fill the axoplasm.

These findings suggest that, in moderate to severe TBI, CMSP occurs and impacts upon concomitant cytoskeletal change. While these studies further implicate $Ca^{2+}$ in the demise of severely injured axons, they do not imply an all or none effect, rather they show evidence for progressive change that may be amenable to rapid therapeutic intervention. This work is supported by grants NS 20193 and The Martin Rodbell Fellowship.

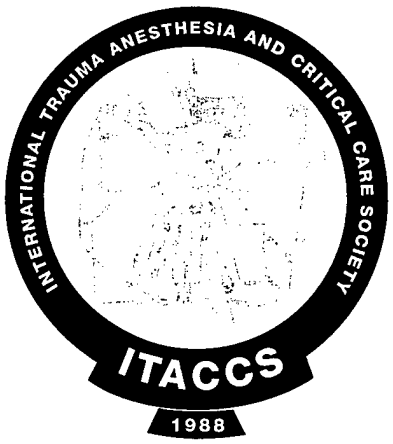
# HYPOTHERMIA IN TRAUMA~

## DELIBERATE OR ACCIDENTAL

Based on a special seminar held in Baltimore at Trauma Care '97, 10th Annual Trauma Anesthesia and Critical Care Symposium and World Exposition, May 15 -17, 1997

The International Trauma Anesthesia and Critical Care Society (ITACCS) is accredited by the Accreditation Council for Continuing Medical Education (ACCME) for physicians. This CME activity was planned and produced in accordance with the ACCME Essentials. ITACCS designates the CME activity for 5 credit hours in Category 1 of the Physicians Recognition Award of the American Medical Association.

CME Questions Included



# Therapeutic Hypothermia After Traumatic Brain Injury or Hemorrhagic Shock: From Mild Cooling to Suspended Animation

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## Objectives:

1. To familiarize the reader with contemporary studies on the application of resuscitative hypothermia in the treatment of traumatic brain injury and hemorrhagic shock.
2. To describe the potential mechanisms for the beneficial effects of hypothermia in these settings.
3. To present some recent findings from both laboratory and clinical studies of resuscitative hypothermia conducted at the University of Pittsburgh.
4. To discuss possible side effects and limitations of the application of therapeutic hypothermia.
5. To discuss future directions for novel applications of hypothermia in combination with pharmacologic interventions.

## Historical Perspective

One of the earliest reports of the potential beneficial effects of hypothermia in the treatment of traumatic brain injury was described by Charles Phelps in 1897 in his classic textbook Traumatic injuries of the brain and its membranes! It is fitting that this monograph was assembled on the 100th anniversary of this remarkable description.

> *"The shaving of the head, which had been advised as a means of facilitating diagnosis, is at the same time a measure of treatment... The essential advantage... to be derived from this procedure is that it permits the effective application of the ice-cap, which next to trephination, ...is most nearly a directly curative resource... It is contraindicated in hemorrhages and cerebral lacerations when uncomplicated by serious contusion; but, as those lesions are constantly thus complicated, it may be held a proper resort when such symptoms are manifest, without regard to exact diagnosis."*

In the early 1940s, Fay[2,3] examined the deliberate application of hypothermia in traumatic brain injury, and this was followed by several additional series of case reports and uncontrolled trials between 1943 and 1979 by other pioneers in this field including Woringer et al,[4] Sedzimir,[5] Lazorhes and Campan,[6] and Rosomoff[7] in traumatic brain injury, Albin et al[8] in spinal cord injury, Bigelow et al[9] and Swan et al[10] in cardiothoracic surgery, Rosomoff et al[11] in focal cerebral ischemia, Siebke et al[12] and Conn et al[13] in near drowning, Wolfe,[14] Benson et al,[15] Ravitch and Safar[16] in cardiopulmonary arrest, and Rush et al[17] in the application of deep hypothermia for total circulatory arrest. Although remarkable effects were suggested in many of these reports, they failed to demonstrate convincingly that hypothermia was beneficial and did not result in the widespread application of resuscitative hypothermia. These reports were complicated by a number of difficulties including variation in depth and duration of hypothermia, and failure to include concurrent normothermic controls. In addition, reports of potential infectious complications in patients treated with the sustained application of moderate hypothermia[18] tempered enthusiasm for further studying resuscitative hypothermia in a controlled fashion.

## Laboratory studies supporting the application of therapeutic hypothermia in traumatic brain injury and hemorrhagic shock

In the mid 1980s there was renewed interest in the laboratory investigation of the deliberate application of therapeutic hypothermia for protection (induced before the insult) or resuscitation (induced after the insult). This work was focused predominantly in models of global cerebral ischemia in rats and monkeys,[19-23] cardiopulmonary arrest[24-28] and near drowning in dogs.[29] Central to this resurgence in interest in hypothermia was the development of three novel concepts: 1) that remarkably mild hypothermia (a temperature reduction of between 3° and 5°C) was effective in reducing secondary brain damage,[19-30] 2) that the duration of mild hypothermia necessary for a beneficial effect might be transient - as short as 1 or 2 hours[19,28] and 3) that brain temperature, not body temperature, was the critical therapeutic target.[19] The chance discovery of the efficacy of mild, transient hypothermia in these studies revived the importance of hypothermia research because mild and transient hypothermia are safer and easier to induce than the previously tried moderate, sustained hypothermia. It is important to define the approximate temperature ranges commonly used to describe specific depths of therapeutic hypothermia. Generally accepted definitions of these ranges are mild (34° to 36°C), moderate (28° to 32°C), deep (15° to 25°C), and profound (< 15°C) hypothermia.[31]

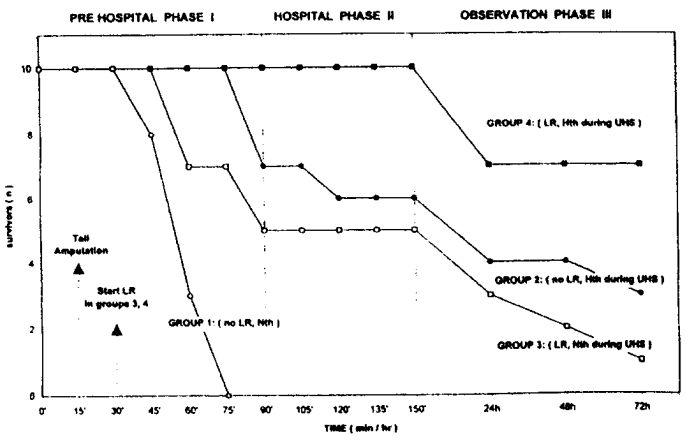


*Figure 1: Survival after uncontrolled hemorrhagic shock (UHS) in rats from the study of Kim et al.[41] The insult in all groups is comprised of a volume controlled initial hemorrhage followed by tail amputation. Treatments include normothermia (Nth, Group 1), hypothermia (Hth, Group 2, 30°C applied between 15 min. and 120 min.), normothermia plus lactated Ringers (LR) fluid resuscitation (Nth + LR, Group 3), or hypothermia plus fluid resuscitation (Hth + LR, Group 4.) Survival to 72 hours was maximal in rats treated with hypothermia plus LR. Reprinted from the Journal of Trauma with permission.*

Specific investigation of the application of therapeutic hypothermia in the treatment of traumatic brain injury was renewed by the report of Clifton et al[32] who observed an inverse correlation between functional outcome and brain temperature (between 30° and 40°C). This was followed by a series of reports from several laboratories further defining the beneficial effect of hypothermia in a wide variety of models (both rodent and canine) of traumatic brain injury.[33-37]

Recent controlled laboratory studies of the utility of resuscitative hypothermia in models of hemorrhagic shock developed from the initial work of Crippen et al in our center[38] and of Meyer and Horton.[39] This resuscitative effect was demonstrated in models of both controlled[38,40] and uncontrolled[41] hemorrhagic shock (Figure 1), and with both mild and moderate hypothermia.[42,43] In controlled laboratory studies addressing an additional hemorrhagic shock-related application of deliberate hypothermia, Tisherman et al[44,45] investigated the application of deep and profound hypothermic circulatory arrest to enable resuscitative surgery that would otherwise be impossible. Our series of studies into "suspended animation" has culminated so far in the study by Capone et al[46] who reported complete recovery of the brain in dogs after normothermic hemorrhagic shock of 1 hour followed by profound hypothermic circulatory arrest of 1 hour. This application of resuscitative hypothermia is being further developed as a possible novel therapeutic approach to the management of pulseless battlefield casualties, specifically, "suspended animation" for transport and repair of otherwise lethal extracranial wounds. "Suspended animation" could be induced and reversed by portable cardipulmonary bypass[47] and followed by subsequent delayed resuscitation.[48]

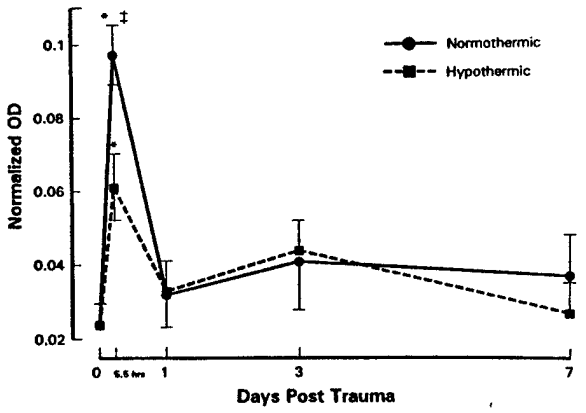


*Figure 2. Desitometric analysis of RNA gel blot hybridizations for IL-1β message before, and at serial times after experimental cerebral contusion in rats, from the work of Goss et al.[46] Filled circles represent data from rats maintained at brain temperature 37°C, while solid squares represent data from rats maintained at a brain temperature of 32°C for 4 hours after injury. A marked increased in IL-β message was observed at 5.5 hours after injury which was partially attenuated by hypothermia. Reprinted from the Journal of Neurotrauma with permission.*

## Why hypothermia: Proposed mechanisms for the beneficial effects of deliberate hypothermia in traumatic brain injury and hemorrhagic shock

Laboratory and clinical trials in cerebral resuscitation from ischemic or traumatic brain injury have repeatedly highlighted the tremendous challenge involved in demonstrating reproducible efficacy, in a wide variety of injury models or injury types, when a single therapeutic agent is used.[49-51] The complex, multifactorial nature of the cascades of secondary damage purported to occur in both ischemic and traumatic brain injury strongly suggests the need for multimodal therapies.[23,48-52] A similar multifactorial pathogenesis is proposed in the evolution of visceral damage after hemorrhagic shock.[31] A great deal of evidence suggests that hypothermia favorably and simultaneously influences a large number of secondary injury mechanisms including; energy failure,[53] oxidant injury,[54,55] delayed neuronal death,[19,36] excitotoxicity,[56] intracranial hypertension[37] edema formation,[35,57] cytoskeletal protein degradation,[58] blood-brain barrier permeability,[59] IL-1β production[60] (Figure 2), and neutrophil accumulation.[61] It is very likely that some critical combination of beneficial effects on these mechanisms is responsible for the success of therapeutic hypothermia in experimental and clinical trials.

## Clinical investigation of therapeutic hypothermia in traumatic brain injury

Although there is a much larger body of laboratory data supporting the use of mild, transient, resuscitative hypothermia in ischemic rather than traumatic brain injury, clinical application of deliberate hypothermia has been spearheaded in controlled trials after traumatic brain injury. Uncontrolled trials of moderate hypothermia in patients after traumatic brain injury looked promising[57,62] but were abandoned because of management problems. Marion et al[63] reported a beneficial effect of moderate (32°C), transient (24 hours) hypothermia on intracranial hypertension in adults with severe closed head injury. A reduction in the need for other therapies for control of intracranial hypertension was observed. Clifton et al[64] reported a reduction in the incidence of posttraumatic seizures in adults treated with moderate hypothermia for 48 hours after severe head injury. A trend toward improved outcome was also observed. Similarly, Shiozaki et al[65] reported efficacy of mild hypothermia in controlling refractory intracranial hypertension in patients with severe traumatic brain injury. Most recently, Marion et al[66] demonstrated that moderate (32°C), transient (24 hours) hypothermia improved functional outcome as measured with the Glasgow outcome scale at 6 months after severe traumatic brain injury in 82 patients randomized to either hypothermia or normothermia. This beneficial effect extended to 12 months in the subgroup of patients with admission Glasgow coma score of 5 to 7 (Table 1). In addition, reductions in IL-β and glutamate concentrations were demonstrated in cerebrospinal fluid samples from hypothermic vs normothermic patients, suggesting the possibility of beneficial effects of hypothermia on posttraumatic inflammation and excitotoxicity, respectively. Remarkably, a significant reduction in cerebral metabolic rate for oxygen was not observed,[63,66] suggesting that this beneficial effect was not due to a simple reduction in cerebral oxidative metabolic demands. A multicenter randomized controlled clinical trial of 48 hours of hypothermia vs normothermia in the treatment of human head injury is currently underway.

## Potential limitations and complications of the application of deliberate hypothermia

Hypothermia is associated with potentially limiting side effects. Suppression of acute inflammation[67] and an increased infection risk[15,18] are concerns. These complications appear to be importantly related to the duration of hypothermia and the underlying condition that is being treated. In traumatic brain injury, Marion et al[66] and Clifton et al[64] did not observe increases in the incidence of infection with 24 hour and 48 hour

**TABLE 1 GLASGOW OUTCOME SCORES IN THE HYPOTHERMIA AND NORMOTHERMIA GROUPS AT 3, 6, AND 12 MONTHS**

| Glasgow Outcome Scores | At 3 Months | | At 6 Months | | At 12 Months | |
|---|---|---|---|---|---|---|
| | Hypothermia | Normothermia | Hypothermia | Normothermia | Hypothermia† | Normothermia |
| **All Patients** | | | | | | |
| 1. (Death) | 8 (20) | 9 (21) | 8 (20) | 10 (24) | 9 (23) | 10 (24) |
| 2. (Vegetative state) | 6 (15) | 11 (26) | 3 (8) | 7 (17) | 3 (8) | 8 (19) |
| 3. (Severe disability) | 11 (28) | 5 (36) | 7 (18) | 11 (26) | 3 (8) | 8 (19) |
| 4. (Moderate disability) | 8 (20) | 4 (10) | 7 (18) | 8 (19) | 9 (23) | 5 (12) |
| 5. (Mild or no disability) | 7 (18) | 3 (7) | 15 (38) | 6 (14) | 15 (38) | 11 (26) |
| Total | 40 | 42 | 40 | 42 | 39 | 42 |
| P Value‡ | 0.12 | | 0.05 | | 0.18 | |
| **Patients with coma score 5 to 7** | | | | | | |
| 1. (Death) | 2 (9) | 5 (19) | 2 (9) | 6 (23) | 2 (9) | 6 (23) |
| 2. (Vegetative state) | 2 (9) | 7 (27) | 1 (5) | 3 (12) | 1 (5) | 4 (15) |
| 3. (Severe disability) | 6 (27) | 9 (35) | 3 (14) | 8 (31) | 3 (14) | 6 (23) |
| 4. (Moderate disability) | 6 (27) | 3 (12) | 4 (18) | 6 (23) | 5 (23) | 2 (8) |
| 5. (Mild or no disability) | 6 (27) | 2 (8) | 12 (55) | 3 (12) | 11 (50) | 8 (31) |
| Total | 22 | 26 | 22 | 26 | 22 | 26 |
| P Value‡ | 0.01 | | 0.01 | | 0.04 | |

*Percentages may not add to 100 because of rounding
†One patient was lost to follow-up
‡P values are comparisons of all five outcomes in the hypothermia and normothermia groups.
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applications of hypothermia, respectively. However, longer applications of hypothermia may have considerable risk.[18] In addition, application of mild hypothermia in settings not associated with ischemia but associated with considerable infection risk (such as elective abdominal surgery in patients with malignancies) increases infection rates.[68]

Coagulopathy is suggested as another potential complication of hypothermia. However, in the studies of severely head injured patients by Marion et al,[63,66] platelet counts and prothrombin times did not differ significantly between groups, and no difference in posttrauma intracranial hematomas or other hemorrhagic complications were noted despite the fact that some of the patients had multiple trauma. Cardiac arrhythmias were also not observed. The threshold for these complications appears to be temperatures below 30°C.[69,70] On the other hand, a recent report[71] suggested that morbid cardiac events after non-cardiac surgery were more common in mildly hypothermic patients compared to those who remained normothermic.

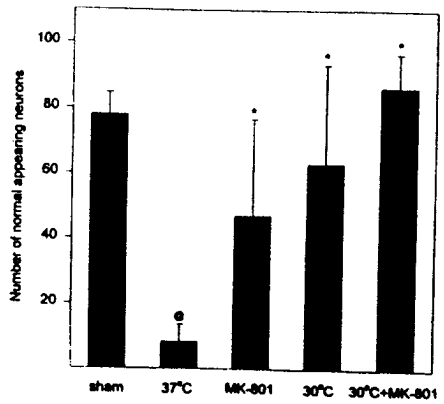


*Figure 3. Bar graph from the work of Dietrich et al[75] showing the number of normal appearing neurons in striatum at 2 months after sham operation or cerebral ischemia in rats treated with normothermia (37°C), the glutamate receptor antagonist MK-801, hypothermia (30°C), or the combination of hypothermia plus MK-801. Neuronal survival was maximal after treatment with the combination of moderate hypothermia and MK-801. Reprinted from the Journal of Cerebral Blood Flow and Metabolism with permission.*

Although the systemic complications appear relatively minimal for the transient (24 hour) application of mild or moderate hypothermia, one area of investigation that deserves further study is that of the effect of hypothermia on regenerative and endogenous defense mechanisms in brain. Goss et al[60] reported that 4 hours of moderate hypothermia resulted in a sustained inhibition of nerve growth factor production in brain after experimental contusion in rats. Nerve growth factor is an important homeostatic molecule in the central nervous system that upregulates antioxidant defenses and prevents apoptosis. The ramifications of this effect of hypothermia on brain parenchyma is currently under investigation.

Finally, another potential limitation of resuscitative hypothermia may be that it produces a temporary rather than sustained effect —i.e., delays rather than ameliorates damage. This possibility was first suggested in classic studies of the effect of hypothemia on acute inflammation,[67,72] and was reintroduced in work by Dietrich et al[73] in models of global cerebral ischemia, where brief episodes (1-3 hours) of hypothermia only delayed death of neurons in selectively vulnerable brain regions. Recent work by Colbourne et al,[74] however, suggests that longer durations of hypothermia may produce permanent benefit.

## Future directions

Some of the most intriguing recent work in the therapeutic application of hypothermia in laboratory studies involves the combination of hypothermia with other therapies. Dietrich et al[75] reported that combination of 3 hours of moderate hypothermia with sustained administration of the glutamate antagonist MK-801 produced a synergistic beneficial effect on neuronal survival in a model of global cerebral ischemia (Figure 3). Similar reports have been suggested for the combination of hypothermia and other therapies.[76] Additional promising strategies that will require further study include the combination of hypothermia with either growth factors,[60] anti-inflammatory agents or flow promoting treatments.[60,77]

*Acknowledgement*

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